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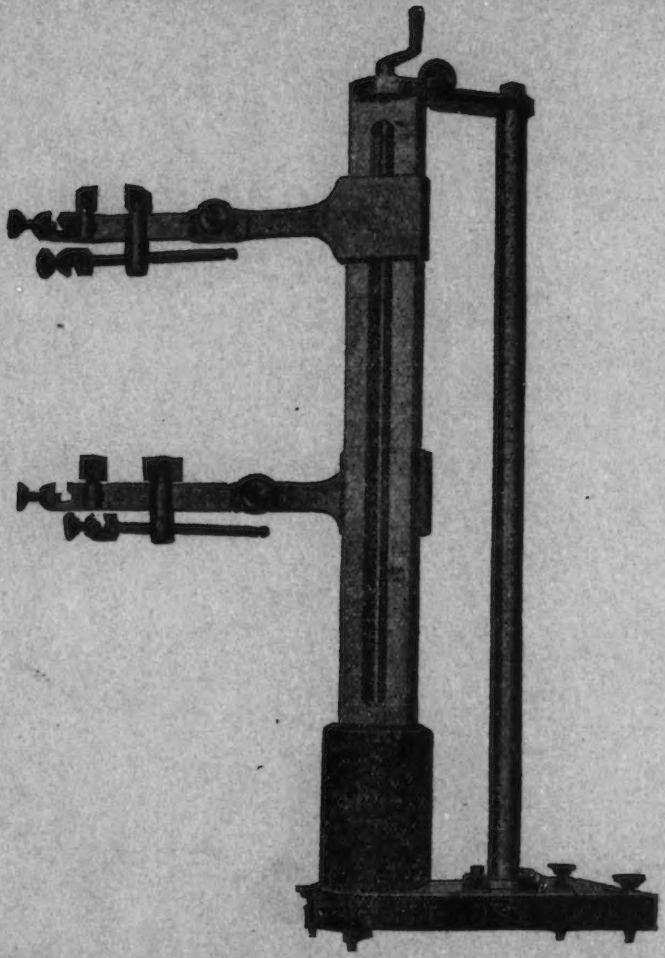

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STUDIES IN THE PHYSIOLOGY OF VITAMINS[1]

VII. Hemoglobin, Solids, Sugar and Chloride Changes in the Blood of Vitamin B Deficient Dogs[2]

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From the Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.

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The majority of blood studies in B-avitaminosis reported in the literature were conducted on rats and pigeons (Funk and von Schönborn, 1914; Mattill, 1923a; Eggleton and Gross, 1925; Drummond and Marrian, 1926; and many others). Some investigators employed dogs as experimental animals but they used diets deficient in more than one of the familiar vitamins (Collazo, 1922–23; Shinoda, 1924). This fact was subsequently recognized by other workers (Bickel, 1924; Tscherkes, 1926). Very few data are available from experiments on dogs in which the diets were complete in every known factor except with respect to vitamin B.[4] During the course of an investigation concerning the physiology of vitamin B in the dog (Stucky, Rose and Cowgill, 1928) observations on certain phases of blood chemistry were made. These preliminary experiments yielded data which in our estimation warranted further study of this problem. This paper is a complete report of these additional studies. Two series of experiments were performed.

Series I. *General technique.* Dogs were used in all the experiments reported in this paper. The animals were placed under observation for

[1] Other papers in this series have appeared as follows: I. This Journal, lviii, 131 (1921); II, Ibid., lxvi, 164 (1923); III, Ibid., lxxiii, 106 (1925); IV, Ibid., lxxvii, 389 (1926); V, Arch. Path., vii, 197 (1929); VI, This Journal, lxxxvii, 85 (1928).

[2] The data in this paper are taken from dissertions submitted by Charles J. Stucky and William B. Rose in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Yale University, 1927 and 1928, respectively.

[3] Porter Fellow of the American Physiological Society, 1926–27.

[4] The term vitamin B as used in this paper refers to the combination of two or more factors which have been shown to make up this substance (Smith, 1928).

at least two weeks before the experiments were begun. During this period attention was paid to their general activity, their desire to eat the artificial food mixture and their behavior in other respects. Only active animals in good nutritive condition, and which appeared particularly well adapted for these experiments were chosen. The animals were kept in individual cages and were bathed regularly. The general procedure for metabolism experiments described by Cowgill (1923a) was employed.

The dogs were fed an artificial food mixture sufficient to maintain a constant body weight. The casein diet III described by Cowgill (1923b) was used. Cod-liver oil was administered at frequent intervals to insure an adequate supply of vitamins A and D. Water was furnished ad libitum. This diet was complete in every known respect except for vitamin B. Each animal was given 15.3 grams of the food mixture per kilo of body weight calculated on the basis of the initial weight. This quantity was offered daily in one portion throughout the course of the experiment, regardless of whether the dog had eaten all of the previous day's allotment or not. During the preliminary observation period each animal was given 0.6 gram of vitavose[5] per kilo of body weight per day (Cowgill, Deuel and Smith, 1925). The vitamin preparation was fed separately from the rest of the ration in order to avoid any possible influence on the palatability of the artificial food mixture.

When rapid recovery from symptoms of B-avitaminosis was desired various preparations of vitamin B were employed as therapeutic agents. These included vitavose, vitamin powder (Harris),[6] vegex[7] and tomato juice.

Eight dogs were used in this first series of experiments. All but one of the animals were fed the basal vitamin B deficient ration described above. One dog served as a normal control and was placed on the basal ration plus 0.6 gram of vitavose per kilo of body weight per day. Two of the animals on the deficient diet had simple gastric fistulae and were used for other experiments, in addition to those herein described.

Blood analyses. The blood was always drawn in the morning when the animals were in a fasting condition. It was usually obtained by venepuncture, and occasionally from the heart. Blood samples were taken about once weekly during the first few weeks of the vitamin B deficient régime but more frequently when polyneuritic symptoms were manifest and during the recovery period after vitamin B therapy. Analyses were made in duplicate on oxalated whole blood in all cases. Hemoglobin, total dry solids, sugar and chlorides were determined.

[5] Obtained through the courtesy of the Ward Baking Company, New York, N. Y., and E. R. Squibb and Sons, New York, N. Y.

[6] From the Harris Laboratories, Inc., Tuckahoe, N. Y.

[7] This is a highly concentrated vegetable extract made from grain grown yeast and prepared with all the salts of sea water. It may be obtained from the Vitamin Food Co., Inc., Westfield, Mass.

Hemoglobin was estimated colorimetrically using a solution of ferric and chromic sulphates as a standard (Haskins and Osgood, 1923). The average of many readings by two observers on duplicate samples was taken as the final value. Percentages are expressed according to the Haldane scale (1900–01).

TABLE 1

Some changes in the blood of a fistula dog during vitamin B deficiency and recovery

Dog 6. October, 1926, to January, 1927. Short-haired female; simple gastric fistula; initial body weight 10.6 kilos.

NUMBER OF DAYS ON DIET	HEMOGLOBIN	TOTAL SOLIDS	SUGAR	CHLORIDES AS NaCl	REMARKS
	per cent	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
11	92		107		
19	96	21.2	95		Appetite good
27	97	20.4	97	433	
32	97	19.5	99	445	Partial anorexia
38	112	23.6	103	456	Partial anorexia
44	96	19.1	143	456	Partial anorexia
48	87	19.6	103	445	Considerable leakage from gastric valve
52	83	17.7	108	433	
59	87	19.1	114	445	Anorexia continues
67	83	18.0	109	445	
73	64	16.3	114	468	
76	68		121	480	
77	73	16.1	101	480	"Polyneuritic" symptoms; body weight 8.9 kilos
			Vitamin B therapy		
3	69	15.4	100		Condition improved
5	65		115	514	Has consumed all food offered; increased water intake
6	55	15.1	126	526	Apparently slowly recovering from vitamin B deficiency
12	69	15.5	116	526	Animal quite active

Estimation of the total solids of the blood was made by pipetting 0.5 to 1 cc. of blood into a small aluminium dish, weighing and drying to constant weight in an electric oven at 105°C.

Sugar was determined by the micro technique of Hagedorn and Jensen (1923). Only 0.1 cc. of blood is required for a single estimation. This method has been found reliable by many investigators (Cori and Cori, 1924–25; Jonsell et al., 1926; Duggan and Scott, 1926; Drummond and Marrian, 1926; Pucher and Finch, 1928; and others).

In estimating the chlorides of the whole blood Van Slyke's modification (1923) of the Whitehorn method (1920–21) was employed. The values are recorded as milligrams of sodium chloride per 100 cc. of blood.

Experimental results. Tables 1, 2 and 3 are representative of the data obtained in the first series of experiments. Blood sugar values are slightly erratic but there is no evidence of a persistent hyperglycemia such as has been reported by Collazo (1922–23) who used pigeons, guinea pigs and dogs as experimental animals. His diets, however, were lacking in more than one of the vitamins.

TABLE 2

Changes in the blood of a dog in vitamin B deficiency

Dog 9. October to December, 1926. Short-haired female; initial body weight 13.0 kilos.

NUMBER OF DAYS ON DIET	HEMOGLOBIN	TOTAL SOLIDS	SUGAR	CHLORIDES AS NaCl	REMARKS
	per cent	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
10	117		102	444	
27	112	22.0	99	445	
32	109	21.2	113	432	Has consumed all food offered to date
38	113	22.1	94	445	Partial anorexia manifested on 37th day
44	114	21.9	102	432	Partial anorexia continues
46	107	21.5	104	421	Vomitus found in cage; complete anorexia until death
48	114	23.4	89	409	
52	121	22.5	94	386	
58	154	25.2	110	328	Severe "polyneuritis;" animal very weak
59	150	26.5	109	281	Body weight 10.8 kilos; convulsions followed by death

The two fistula animals used in these experiments developed anemia during the progress of B-avitaminosis which was not ameliorated by vitamin B therapy. This is clearly seen from an inspection of the values for hemoglobin and total solids of dog 6 (table 1). It is possible that the presence of the gastric fistula may have been responsible in part, at least, for the anemia that developed.

In the case of dog 12, vitamin B therapy was followed by a pronounced drop in the hemoglobin and total solids (table 3). The normal control animal, receiving the same diet plus an adequate supply of vitamin B (vitavose), yielded fairly constant values for the blood constituents studied throughout the course of the experiment (80 days). In view of these

results it appeared highly desirable to determine whether anhydremia was a constant phenomenon associated with vitamin B deficiency in non-fistula dogs. Moreover, it is conceivable that the values for other blood constituents (e.g., the chlorides) may be influenced by pronounced changes in blood concentration (table 2).

In observing the animals from day to day, the impression was gained that there was a diminished water consumption during vitamin B deficiency. This appeared more significant to us when some evidence was forthcoming

TABLE 3

Changes in the blood of a dog in vitamin B deficiency and recovery

Dog 12. December, 1926, to February, 1927. Short-haired female; initial body weight 6.4 kilos.

NUMBER OF DAYS ON DIET	HEMOGLOBIN	TOTAL SOLIDS	SUGAR	CHLORIDES AS NaCl	REMARKS
	per cent	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
7	88	18.6	105	445	
15	90	19.4	92	433	Consumed all food offered during the first ten days
21	92	19.4	114	445	Partial anorexia since 11th day
31	96	20.2	109	445	Animal quite active
37	114	21.3	102	433	Water intake decreased
45	116	21.8	90	409	Partial anorexia continues
47	120	22.0	114	456	Complete anorexia; animal less active
48	125	22.0	114	456	Neuro-muscular symptoms
			Vitamin B therapy		
4	98	18.6	100	468	Condition improved
8	93	18.4	97		Animal more active
15	93	19.1	89		Water intake increased since vitamin B therapy was begun
25	103	19.2	96		Animal apparently normal again; body weight 6.2 kilos; experiment discontinued

that anhydremia may be associated with this deficiency disease. Consequently, a second series of experiments was begun, employing only non-fistula dogs and rigidly controlling their water consumption

SERIES II. *General technique.* In preparing animals for these experiments the general procedure described for series I was followed. However, blood analyses were also made during a preliminary observation period. Only those dogs which showed normal values for the blood constituents studied over a period of several weeks and appeared normal in other

respects were selected for the experiments. Eleven animals were chosen and separated into two groups. One group of seven received the basal vitamin B deficient ration previously described. Water was furnished ad libitum. The other group of four dogs acted as *food and water controls.* They were carefully paired with four dogs in the vitamin B deficient group, special attention being paid to size, weight, stature and general activity. Dogs 21, 22, 23 and 24 served as the controls of the vitamin B deficient dogs 25, 26, 27 and 28 respectively. *Each control was given the same amount of food and water that was consumed by the corresponding vitamin B deficient dog.* In addition, each control dog was given a supplement of 0.6 gram of vitavose per kilo of body weight per day, regardless of its daily food allotment. Water and food pans were suspended securely in the cages in order to prevent any loss by spilling. By means of a control water pan placed in the animal room, the amount of water evaporated per day was noted. Due allowance was made for this factor in recording the quantity of water consumed by the deficient animals. In this manner any possible effect of flucutations in food and water consumption on the blood constituents during B-avitaminosis was controlled. The control dogs were started on the experiments two days later than the animals in the vitamin B deficient group.

Blood analyses. Blood samples were obtained by cardiac puncture only. The sugar, chlorides and total solids of the blood were estimated as in the first series of experiments. Hemoglobin was determined colorimetrically according to a modification of the Cohen and Smith technique (1919). The acid hematin standard was checked at frequent intervals and prepared fresh whenever required. Duplicate determinations were made in all cases.

Definition of the term "period" as used in tables. The length of time required to produce severe symptoms of vitamin B deficiency in dogs on the same diet varied approximately from four to ten weeks. In order to correlate the results obtained from a series of dogs, it was deemed advisable to separate the data obtained into five "periods" corresponding more or less closely to various stages of vitamin B deficiency and recovery. The first three periods, namely, periods I, II and III represent stages during the course of vitamin B deficiency. The last two periods, namely, periods IV and V indicate stages during realimentation.

It must be remembered that this subdivision into periods is purely an arbitrary approximation based on the following observations.

Period 1 represents the interval during which the animal appeared essentially normal in every respect.

Period 2—intermediary period—characterized by some decrease in general activity, partial anorexia and a variable loss in body weight.

Period 3—the advanced stage of B-avitaminosis—a decrease in general

TABLE 4

*Some blood changes and the food and water consumption of vitamin B deficient dogs and their corresponding controls**

FOOD AND WATER CONSUMPTION				BLOOD CHANGES†							
Dogs 25 and 21				Dogs 25 (Vitamin B Deficient)				Dog 21 (Control of Dog 25)			
Period‡	Duration of period	Average daily food intake	Average daily water intake	Hemoglobin	Total solids	Sugar	Chlorides as NaCl	Hemoglobin	Total solids	Sugar	Chlorides as NaCl
	days	*grams*	*grams*	*per cent*	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
1	15	140	390	116	21.5	89	486	111	20.3	87	493
2	21	58	217	117		80	481	123		97	482
3	13	0	135	127	22.0	88	456	132	22.5	112	456
Realimentation (Dog 21)				Animal died on 50th day of vitamin B deficiency; hemoglobin at death = 133 per cent				Realimentation			
4	16	228	610					105	20.2	105	502
5	5	140	546					100		110	497
Dogs 26 and 22				Dog 26§ (Vitamin B Deficient)				Dog 22 (Control of Dog 26)			
1	29	120	279	111	20.4	89	499	115	21.6	102	505
2	29	71	199	107	19.7	90	494	114	21.2	106	495
3	9	52	241	111	20.8	97	450	125	22.6	98	464
				Realimentation							
4	5	140	495	90	17.4	102	503	118	21.2	96	486
5	10	230	410	96	18.8	97	510	109	20.8	98	483
Dogs 27 and 23				Dog 27 (Vitamin B Deficient)				Dog 23 (Control of Dog 27)			
1	28	165	450	120	22.1	104	488	113	21.0	102	507
2	20	70	308	113	20.1	110	464	112	20.8	102	514
3	7	0	172	120	20.4	88	437	120	20.8	86	509
				Realimentation							
4	12	163	630	82‖	17.5‖	107	522	105	20.3	89	491
5	5	165	242	109	20.0	105	497	108	20.4	94	493

* Dogs 21, 22, 23 and 24 (controls) were given 0.6 gram vitavose (vitamin B preparation) per kilo of body weight per day in addition to a quantity of food and water equivalent to that consumed by dogs 25, 26, 27 and 28 (vitamin B deficient) respectively.

† The values for the blood constituents represent the averages of several estimations in practically all cases.

‡ For explanation of "period" see text.

§ Vitamin B therapy begun 67th day of vitamin B deficiency.

‖ An infection (B. welchii) developed at the site of the yeast (Harris) injection. Hence the drop in the hemoglobin and total solids may have been due in part to this factor. On the 63rd day the hemoglobin dropped to 77 per cent but then rose to 86 per cent on the 68th day, when the infection had cleared up.

TABLE 4—*Concluded*

FOOD AND WATER CONSUMPTION				BLOOD CHANGES†							
Dogs 28 and 24				Dog 28 (Vitamin B Deficient)				Dog 24 (Control of Dog 28)			
Period‡	Duration of period	Average daily food intake	Average daily water intake	Hemoglobin	Total solids	Sugar	Chlorides as NaCl	Hemoglobin	Total solids	Sugar	Chlorides as NaCl
	days	*grams*	*grams*	*grams*	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
1	27	200	501	118	20.6	92	503	104	19.2	104	515
2	31	142	395	119	21.9	95	476	104	19.3	109	520
3	8	115	543		23.4	94	442	100	19.5	95	497
Realimentation (Dog 24)				Died on 67th day of vitamin B deficiency; ante-mortem values: total solids 24.2 per cent; sugar 96 mgm. per 100 cc.; chlorides 429 mgm. per 100 cc.				Realimentation			
4	3	205	1,250					100	19.1	96	498
5	3	205	1,000								

activity, diminished food and water consumption, marked loss in body weight, occasional vomiting, nervous manifestations and changes in some of the blood constituents.

Period 4 represents the immediate effect of vitamin B therapy as indicated by a disappearance of nervous symptoms, pronounced increase in food and water consumption, very definite changes in some of the blood constituents, increase in activity and rapid gain in body weight.

Period 5 is essentially a return to normal as evidenced by restoration of the body weight and appetite and a readjustment of the blood constituents to approximately the normal levels.

Experimental results. A. *Comparison of blood changes in four vitamin B deficient dogs and their corresponding food and water controls.* These data are presented in table 4. A brief inspection of this table will make it evident that the results obtained for the blood sugar for both control and deficient animals fail to show any significant variation from the normal. On the other hand, all the deficient animals manifested a distinct decrease in whole blood chlorides while two of the control dogs failed to reveal any significant change in this blood constituent.

The most striking effect observed in this study was a change in blood concentration as indicated by the hemoglobin and blood solids. As will be seen from table 4, the results obtained are not uniform and can best be presented by considering each individual pair of animals separately.

The increase in blood concentration in deficient dog 25 is comparable to that observed in the corresponding food and water control, dog 21. Both dogs manifested a marked decline in body weight (table 7).

TABLE 6

Changes in the blood and body weight of dogs in vitamin B deficiency

Comparison of initial and final values*

HEMOGLOBIN | TOTAL SOLIDS

TABLE 5

Changes in the blood and body weight of dogs in vitamin B deficiency

Comparison of initial and final values*

DOG NUMBER	HEMOGLOBIN			TOTAL SOLIDS			SUGAR			CHLORIDES AS NaCl			BODY WEIGHT			REMARKS
	Normal	Final	Difference	Normal	Final	Difference	Normal	Final	Difference	Normal	Final	Difference	Normal	Final	Difference	
	per cent	*per cent*		*per cent*	*per cent*		*mgm. per 100 cc.*	*mgm. per 100 cc.*		*mgm. per 100 cc.*	*mgm. per 100 cc.*		*kilos*	*kilos*		
9	115	152	37	22.0	25.9	3.9	101	110	9	445	305	140	13.0	10.8	2.2	Complete anorexia during last two weeks of deficiency
10	121	110	11	24.7	22.1	2.6	97	92	5	456	409	47	11.4	8.3	3.1	Severe polyneuritis. Complete anorexia during last 5 days
11	103	118	15	21.6	22.8	1.2	101	87	14	450	386	64	9.3	7.5	1.8	Consumed all food offered until 2 days before death
12	88	120	32	18.6	21.9	3.3	105	106	1	445	440	5	6.4	5.1	1.3	Complete anorexia for 2 days
13	82	104	22	18.3	20.8	2.5	112	117	5	468	427	41	12.4	8.5	3.9	Complete anorexia during last 2 weeks of deficiency
25	116	127	11	21.5	22.0	0.5	89	88	1	486	456	30	9.3	6.7	2.6	Died of severe polyneuritis on 50th day
26	111	111†	0	20.4	20.8	0.4	89	97	8	499	450	49	8.3	6.3	2.0	Appetite erratic
27	120	120†	0	22.1	20.4	1.7	104	88	16	488	437	51	10.8	9.1	1.7	Complete anorexia during last 18 days of deficiency
28	118	‡		20.6	24.2	3.6	92	96	4	503	429	74	13.2	10.7	2.5	Died with severe polyneuritis on 67th day. Complete anorexia last 4 days
29	127	137	10	22.2			108	92	16	480	439	41	13.5			Severe polyneuritis. Convulsions and death on 39th day of deficiency
30	114	161	47	21.2			78	80	2	507	441	66	15.0			Complete anorexia during last week of deficiency. Died on 40th day
31	106	108†	2	20.1			95	96	1	509			6.4	5.6	0.8	Mild polyneuritis. Complete anorexia during last 6 days of deficiency
Average§...	109	124		21.1	22.3		98	96		475	420		10.1	7.9		
Per cent difference			13.7			5.7			2.04			11.6			21.8	

* Practically all values for the blood constituents in this table represent the averages of estimations covering a period of several days in each case. † A marked reduction in blood concentration occurred as soon as vitamin B therapy was instituted. ‡ Hemoglobin sample lost. The total solids estimation indicates a marked increase in blood concentration. § Averages only for those figures for which the initial and final values are given.

In sharp contrast to the findings in dogs 21 and 25 are those observed in dogs 24 and 28. The food and water control, dog 24, maintained remarkably constant values for both the hemoglobin and total blood solids throughout the entire experiment, whereas the corresponding deficient animal, dog 28, revealed a pronounced increase in the total blood solids. Although dog 28 lost 18.9 per cent of its initial body weight, the corresponding control failed to reveal any decrease in weight in spite of the fact that its caloric intake was the same as that of the deficient animal.

It is evident from table 4 that deficient dogs 26 and 27 revealed a greater reduction in blood concentration (as indicated by the hemoglobin and total solids) during recovery than that obtained in the corresponding controls, dogs 22 and 23.

In general, then, it may be stated that with the exception of one pair (dogs 21 and 25) the deficient dogs all manifested greater changes in blood concentration than their corresponding water and food controls. In the latter instance it would appear that starvation was a significant factor because dog 25 had complete anorexia for the deficient ration for 21 days; consequently, the control dog was starved for the same length of time.

B. *Changes in the blood composition and body weights of twelve dogs during vitamin B deficiency.* The results are summarized in table 5, which includes data obtained from non-fistula dogs in the first series of experiments.

Blood sugar. From a comparison of the blood sugar level in normal dogs with that obtained in the same animals during the advanced stages of vitamin B deficiency it is obvious that only slight changes occurred in all cases.

There are numerous reports in the literature concerning blood sugar studies in vitamin deficiency. Thus, Funk and von Schönborn (1914) reported the occurrence of hyperglycemia in polyneuritic pigeons. An examination of the tables showing the blood sugar values of polyneuritic hens (Thompson and Carr, 1923) indicates that there may be considerable fluctuations in the level of this constituent during the deficient régime; many of the values are within the physiological range and there does not appear to be an initial hypoglycemia and a subsequent hyperglycemia as reported by Collazo (1922–23). These investigators used polished rice as the main ingredient of the ration fed. According to Shinoda (1924), who made extensive studies on the blood sugar of avitaminotic animals, hyperglycemia was not a constant characteristic in polyneuritic birds; thus, in pigeons which had lost weight because of the vitamin free ration, hyperglycemia was absent even when nervous symptoms appeared. This investigator also found, in the case of vitamin-deprived dogs, that the blood sugar level may remain within the physiological range until shortly before death when it suddenly rises. In other cases he obtained results similar to those reported by Collazo (1922–23). It should be noted that

Shinoda and Collazo used diets lacking more than one vitamin; hence their results are not strictly comparable to the data reported in this paper.

Several investigators have used rats in their studies of the blood sugar in vitamin B deficiency. From their observations Eggleton and Gross (1925) concluded that the results indicated nothing abnormal in the deficient rats until the stage of final collapse, when the blood sugar fell to a starvation level. Contrary to Funk's experience with pigeons, there was no evidence of hyperglycemia. On the other hand, Drummond and Marrian (1926) reported that "there appears to be a definite hyperglycemia in the rat during the greater part of the period it is kept on a ration deficient in vitamin B" and that "there is a very marked fall in the blood sugar level during the final stage of decline when the body temperature and metabolism are also reduced."

On the contrary, Racchiusa (1926–27) reported that vitamin B deficiency in the pigeon *is not characterized by an initial hypo- and a subsequent hyper-glycemia; both phenomena may be entirely absent.*

It is worthy of note in this connection that Mattill (1923b) determined the respiratory quotients of rats fed a "synthetic," vitamin B deficient ration. He concluded that vitamin B was "not related specifically to the metabolism of carbohydrates."

As far as the writers are aware no investigations of the blood sugar level in dogs on diets complete except for vitamin B have yet been reported. On the whole, the data presented in this paper indicate that vitamin B does not play a specific rôle in the regulation of the blood sugar level in this species.

Blood chlorides. In all cases the whole blood chlorides were decreased to a greater or less extent during the advanced stages of B-avitaminosis. The average decrease in chlorides was 11.6 per cent. It is not unlikely that the decrease in chlorides observed in vitamin B deficient dogs is due largely, if not entirely, to inanition (Morgulis and Edwards, 1924). No reports concerning the blood chloride level in vitamin B deficiency could be found.

Blood concentration (anhydremia). The average increment in the hemoglobin of all vitamin B deficient dogs (table 5) was 13.7 per cent. This fact is particularly significant because it is well known that a prolonged increase in blood concentration is associated with a destruction of erythrocytes (Marriot, 1923).

In other experiments (to be reported elsewhere) it was found that when dogs were thirsted for only one week a very definite destruction of hemoglobin occurred. This became evident as soon as water was administered. Hence, it is not surprising to note that the average increase in hemoglobin observed in the vitamin B deficient dogs was not greater than 13.7 per cent.

The fact that this value is not an accurate index of the extent of the anhydremia that developed is demonstrated by the data presented in table 6, which represents the effect produced by the administration of vitamin B. Dogs 10, 26 and 27 *apparently* did not show a blood concentration, as determined by increases in hemoglobin or total solids. Nevertheless, vitamin B therapy was followed by a pronounced decrease in both hemoglobin and total solids. *This decrement in blood concentration was a constant finding when vitamin B was administered to a "polyneuritic" animal.* This is clearly evident from the data presented in table 6. The average decrease in the hemoglobin of six dogs was 21.5 per cent. Likewise, the average decrease in the total solids was 15.1 per cent. From these data it would appear that a definite anhydremia exists in dogs in advanced stages of B-avitaminosis, although this condition may be masked by an associated blood destruction. Thus dogs 10 and 27 in which there was some decrease in hemoglobin or solids during vitamin B deficiency showed a marked decrement in these constituents after specific therapy.

An increase in blood concentration which is apparent and marked not infrequently occurs as a terminal phenomenon. Results of this nature were obtained with dogs 9, 25, 28, 29 and 30 (table 5). When this pronounced terminal anhydremia develops, efforts to save the animal are almost invariably unsuccessful with the therapeutic measures at our disposal at present.

In connection with other experiments by one of us (R), the reduction in blood concentration as evidenced by a decrease in hemoglobin was repeatedly confirmed on the same dog after vitamin B administration. The experiments referred to extended over a period of four hundred days.

In view of the fact that the data obtained in two series of experiments with dogs show: 1, an average increase in hemoglobin of 13.7 per cent (table 5); 2, an average increase in blood solids of 5.7 per cent (table 5); 3, a marked reduction in blood concentration in every case following vitamin B therapy (table 6), it is difficult to escape the conclusion that anhydremia to a greater or less degree almost constantly develops when dogs are fed a diet lacking vitamin B. Furthermore, this anhydremia may be completely masked by the associated blood destruction—when determinations of hemoglobin and total solids are employed as the sole criteria of dehydration—and rendered evident only after vitamin B is administered.

It has recently been reported (Sure et al., 1928) that nursing rats in a state of incipient polyneuritis show marked anhydremia. Similar observations were reported by Rose and Stucky (1928), using animals weighing between 200 and 250 grams. However, in the latter experiments it was found that the food and water control rats manifested an increase in blood concentration commensurate with that observed in the deficient group. On the other hand, the increase in blood concentration observed in the

vitamin B deficient dogs could not be fully accounted for in the food and water controls.

It will be seen from table 5 that the average loss in body weight of ten dogs during vitamin B deficiency was 21.8 per cent of their initial weight. Other investigators (Osborne and Mendel, 1917; Karr, 1920; and others) have shown that anorexia occurs in vitamin B deficiency. Other workers have reported that there is an increase in blood concentration in starva-

TABLE 6

*Reduction in the blood concentration of vitamin B deficient dogs by vitamin B therapy**

DOG NUMBER	NORMAL	"POLYNEURITIC"	RECOVERY	DECREASE IN Hb AFTER VITAMIN B THERAPY	
				Difference	Per cent difference
			Hemoglobin per cent		
10	121	110	85	25	22.7
12	88	120	93	27	22.5
13	82	104	83	21	20.2
26	111	111	90	21	18.9
27	120	120	82	38	31.7
31	106	108	94	14	13.0
Average....	105	112	88	24.3	21.5
			Total solids		
10	24.7	22.1	18.8	3.3	14.9
12	18.6	21.9	18.4	3.5	16.0
13	18.3	20.8	17.9	2.9	13.9
26	20.4	20.8	17.4	3.4	16.3
27	22.1	20.4	17.5	2.9	14.2
31	20.1†		17.6†		
Average....	20.8	21.2	18.0	3.2	15.1

* Many of the values in this table represent the averages of a number of determinations made at frequent intervals during the course of this study.

† Not included in the average.

tion. Could we safely attribute the increase in blood concentration in the deficient animals to partial starvation alone? In view of our observation that vitamin B deficient dogs seem to drink less water, is it possible that the anhydremia described is partly due to diminished fluid intake?

In anticipation of these questions water and food control dogs were employed in these experiments (table 4). The water consumption of the vitamin B deficient dogs was carefully measured (table 8). In addition,

a comparison of the body weights of deficient and control dogs is presented (table 7).

With the aid of the data now available an attempt will be made to answer the above questions. From table 7 it is evident that the average loss in body weight of four vitamin B deficient dogs was 21.2 per cent of their initial weight. In striking contrast the average loss of weight in the four food and water control animals was only 5.9 per cent. Hence it would appear that the decrease in caloric intake in vitamin B deficiency due to anorexia can not completely account for the marked loss in body weight in this deficiency disease.

TABLE 7

Apparent lack of economy in the utilization of foodstuffs during vitamin B deficiency

Comparison of the body weights of deficient dogs and their food and water controls

DEFICIENT DOGS*				CONTROL DOGS†			
Dog number	Initial weight	Weight at the end of vitamin B deficiency	Per cent loss in weight	Dog number	Initial weight	Weight at the end of vitamin B deficiency	Per cent loss in weight
	kilos	*kilos*			*kilos*	*kilos*	
25	9.3	6.7	27.9	21	9.3	7.1	22.8
26	8.3	6.3	24.1	22	8.3	8.1	2.4
27	10.8	9.1	15.7	23	9.3	9.1	2.2
28	13.2	10.7	18.9	24	13.6	13.6	0
Average...	10.4	8.2	21.2		10.1	9.5	5.9

* Received diet III (Cowgill, 1923b).

† These animals received diet III supplemented by 0.6 gram of vitavose (vitamin B preparation) per kilo of body weight per day. In addition each control was fed the same amount of food and water that was consumed by the corresponding vitamin B deficient animal. Dogs 21, 22, 23 and 24 were the controls of dogs 25, 26, 27 and 28 respectively.

The data presented in table 8 show that there is a decrease in water consumption along with the anorexia in vitamin B deficiency. However, inasmuch as the control dogs received the same amount of water daily as the deficient animals consumed, the diminished water intake can not entirely account for the greater blood concentration in the deficient animals.

It is worthy of note that the daily intake of water soon after vitamin B therapy was usually far in excess of the normal. During the first period of realimentation (period 4) this amounted to 62.8 per cent above the habitual average water consumption for these dogs (table 8). Likewise, it is of interest that this increase in water consumption during the first period of realimentation was invariably associated with a marked reduction in blood concentration (table 6).

In view of the fact that the deficient animals developed a greater blood concentration and suffered a greater loss in body weight than was observed in the food and water controls, it would appear that the *abnormal* loss in weight in vitamin B deficiency—in excess of the loss due to a diminished caloric intake—may possibly be a factor in accentuating the tendency to anhydremia in the deficient dogs.

Apparently, there is a lack of economy in the utilization of foodstuffs in the deficient animals (table 7) which is difficult to account for. A review of the literature does not reveal any data which would help in the interpretation of this phenomenon. Work bearing on this subject was

TABLE 8

Food and water consumption of dogs during vitamin B deficiency and realimentation

PERIOD*	AVERAGE DURATION OF PERIOD	AVERAGE FOOD INTAKE PER KILO PER DAY†	AVERAGE WATER INTAKE PER KILO PER DAY†	PERCENTAGE OF "NORMAL" FOOD INTAKE	PERCENTAGE OF "NORMAL" WATER INTAKE
Vitamin B deficiency‡					
	days	*grams*	*grams*		
1	19	15.1	39.5	100	100
2	24	7.4	28.7	49.1	72.7
3	8	2.3	24.9	15.2	63.0
Realimentation‡					
4	11	15.1	64.3	100	162.8
5	10	21.6	50.8	143	128.6

* For significance of the term "period" see text.

† These values represent weighted averages of food and water consumed by all dogs.

‡ This table represents data from seven dogs during the course of vitamin B deficiency. Only three dogs recovered after specific therapy was instituted.

reported by Bickel and collaborators (1924–25). However, it must be remembered that they employed diets which were deficient in more than one of the vitamins. Nevertheless, it may be of interest to recall that they reported an increase in the urinary carbon to nitrogen ratio, which Rosenwald (1926) believes to be due, in part at least, to an abnormal elimination of lactic acid in the urine.

SUMMARY AND CONCLUSIONS

Dogs in good nutritive condition, weighing between six and fifteen kilos were fed a basal, "synthetic" ration, complete in every known respect except for vitamin B. Blood studies were made during the progress of B-avitaminosis and during a realimentation period after liberal adminis-

tration of vitamin B. The blood was analyzed for sugar, chlorides, hemoglobin and total solids.

Two series of experiments were performed, employing a total of nineteen dogs. The water and food consumption of the vitamin B deficient animals was measured during the second series of experiments. Four dogs were selected as controls. These were carefully paired with four animals fed the basal vitamin B deficient ration. Each control dog received the same quantity of water and food daily that was consumed by the corresponding animal in the vitamin B deficient group. In addition, each control was given a daily supplement of vitamin B (vitavose).

From the data it appears that the blood sugar values for both vitamin B deficient and control animals show no significant variations from the normal. This indicates that a lack of vitamin B does not affect the regulation of the blood sugar level in the dog.

Blood chlorides tend to decrease during the progress of B-avitaminosis. Similar changes were observed in the water and food control animals. In several cases there was a marked decrement in the chlorides. This was invariably associated with prolonged starvation.

It was found that dogs develop anhydremia in the advanced stages of vitamin B deficiency. This statement is based upon the following observations: 1, an average increase in hemoglobin of 13.7 per cent (eleven dogs); 2, an average increase in total solids of 5.7 per cent (nine dogs); 3, a marked reduction in the blood concentration of "polyneuritic" animals soon after the administration of vitamin B; the decrease in the hemoglobin and total solids of six dogs was 21.5 per cent and 15.1 per cent respectively.

The degree of anhydremia, in most cases at least, appears to be related to loss in body weight. Only one animal (a control) failed to show some evidence of anhydremia. This was the only instance in which no loss in body weight occurred. The corresponding vitamin B deficient dog developed anhydremia and lost weight.

Evidence is presented indicating that a lack of vitamin B is associated with a definite decrease in water consumption. It is impossible to state at present whether this can be completely accounted for by the accompanying anorexia.

Data obtained in these experiments suggest that there is a lack of economy in the utilization of foodstuffs in B-avitaminosis.

In general it appears that the changes observed in the blood constituents here studied of dogs during the progress of vitamin B deficiency may be largely attributed to starvation incident to this deficiency disease. Nevertheless, it seems that lack of vitamin B accentuates the anhydremia which is frequently observed in starvation. Whether this greater tendency to anhydremia in the deficient animals can be attributed entirely to their greater loss in body weight remains unsettled.

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THE EFFECTS ON THE STOMACH OF STIMULATION OF THE DORSAL VAGUS NUCLEI

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The origin of the vagus fibres which influence the motility of the stomach is considered by various authorities to be in the dorsal vagus nucleus. Molhant (1910), Gaskell (1920), Kappers (1920), Herrick (1927) and Ranson (1927) are agreed that the dorsal vagus nuclei give origin to centrifugal fibres which form connections with the motor cells of the enteral system. The literature on the subject is given by Molhant (1910).

The experimental work supporting these views consists mainly of degenerative sections of the vagus nerves and observations for chromatolysis in the bulbar nuclei which belong to them. The effects on the stomach of electrical stimulation of the dorsal vagus nuclei have never been recorded.

The present work shows the effects on the stomach of faradisation of the dorsal vagus nuclei.

METHODS. Cats, which had been starved for twenty-four hours, were used in these experiments. The animal in each experiment was anesthetised with ether and tracheotomy performed. A rubber balloon, attached to a small rubber catheter, was introduced into the stomach through a cervical esophagotomy. The free end of the catheter was attached to a water manometer with thick walled rubber tubing, air transmission being used. The animal was now placed in the prone position the head being supported in a suitable head holder. The spinal cord was cut at the level of the third cervical segment to eliminate possible muscular and vasomotor effects when the stimulus was applied to the nucleus. Artificial respiration was supplied with a pump, the incoming air containing an adequate amount of ether supplied by a Brodie anesthetising apparatus. The floor of the fourth ventricle was now exposed by making an opening through the occipital bone and removal of a portion of the cerebellum. Following the operation the wounds were closed and the animal allowed to recover from shock.

Records of the stomach were taken after about two hours had elapsed. The balloon was inflated, the pressure in the system being 4 to 8 cm. of water. Faradic stimulation was applied over the dorsal vagus nuclei

with a unipolar, platinum or silver electrode. The current was supplied from a Cambridge coil, secondary distance ranging from 185 to 215 mm., the ammeter in the primary showed a current of 0.4 ampere when the vibrator was in action. An indifferent electrode of German silver was

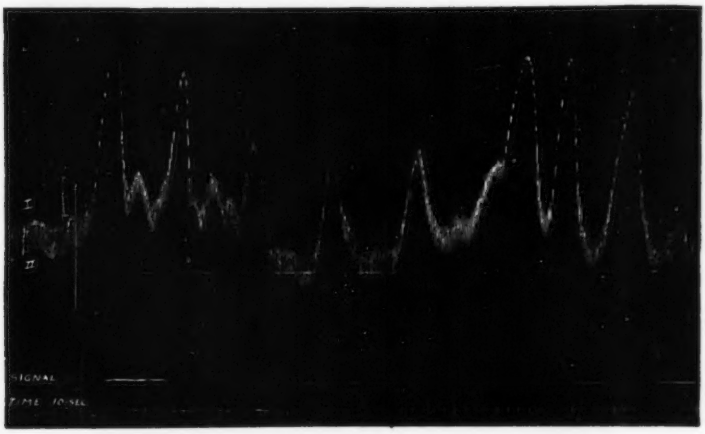

Fig. 1. Effect on the stomach of the cat of faradic stimulation of the cephalic half of the left dorsal vagus nucleus. I, contractions in the stomach; II, entogastric base line.

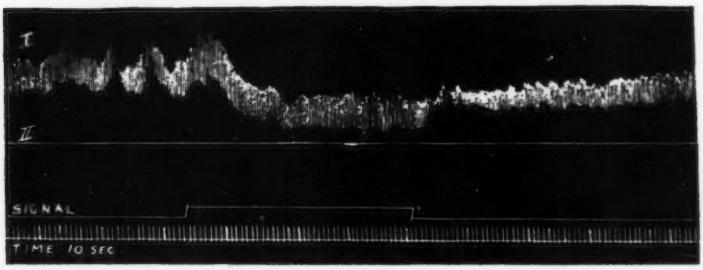

Fig. 2. Effect on the stomach of the cat of faradic stimulation of the cephalic half of the left dorsal vagus nucleus. Stomach hypertonic at the time of stimulation. I, inhibition of stomach tone; II, entogastric base line.

attached to the left foreleg. The effects of stimulation on the stomach were recorded on a smoked drum. Various points immediately adjacent to the dorsal vagus nuclei were stimulated for purposes of control.

In some of the experiments, fluoroscopic examinations and x-ray photographs were made of the stomach during and after stimulation of the

nuclei. In these experiments the stomach was filled with a mixture of milk and barium sulphate about twenty minutes before the stimulus was applied.

In all experiments bristles were inserted in the floor of the fourth ventricle to mark the point where the stimulus was applied. The brains were preserved in formalin and histological studies made using the Weigert hematoxylin method.

OBSERVATIONS. Faradic stimulation applied over the cephalic half of the dorsal vagus nuclei produces, after a definite latent period, marked

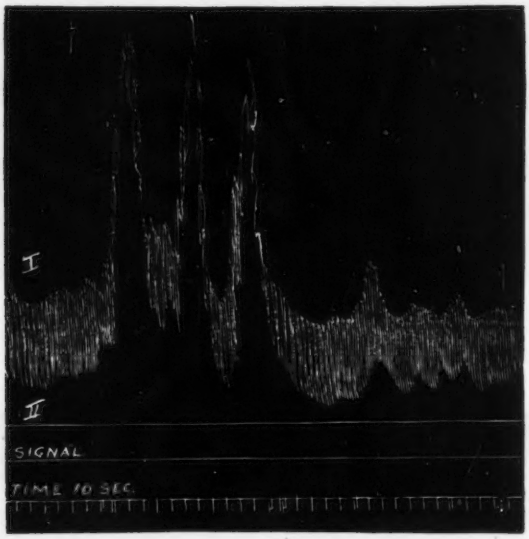

Fig. 3. Effects on the stomach of cat of inserting a bristle into the cephalic portion of the dorsal vagus nucleus. I, contractions in the stomach; II, entogastric base line.

changes in the stomach. The effects from the left nucleus are more pronounced than those from the right nucleus and are obtained with weaker stimuli. In figure 1 powerful contractions of the stomach, resulting from stimulation over the cephalic half of the left dorsal vagus nucleus are shown. If the stomach is hypertonic at the time of stimulation a definite diminution of tone is recorded (fig. 2). Faradic stimulation applied to points immediately adjacent to the cephalic half of the dorsal vagus nucleus and to the caudal portion of the nucleus itself was without effects.

The positions of the bristles in the floor of the fourth ventricle, marking the points of stimulation in the experiment in which figures 1 and 2 were

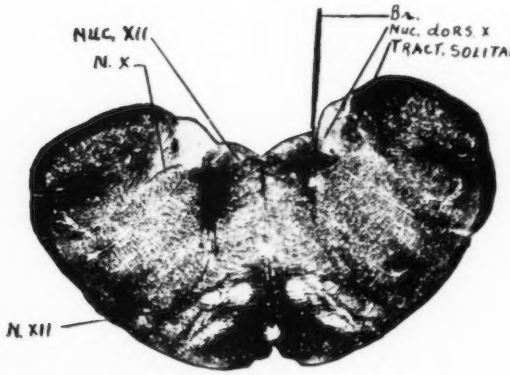

Fig. 5

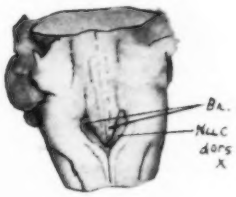

Fig. 4.

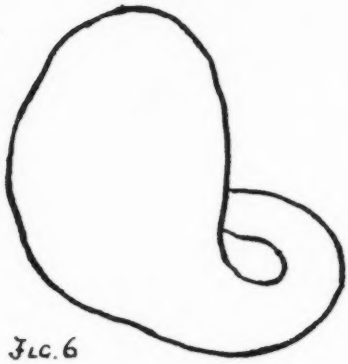

Fig. 6

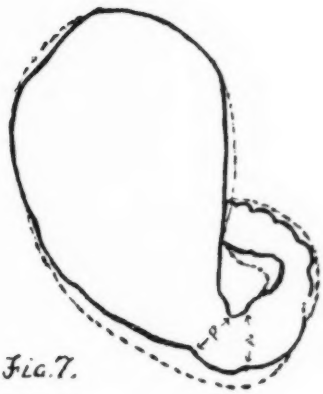

Fig. 7.

Fig. 4. Photograph of a portion of the brain stem (cat) showing bristles in the floor of the fourth ventricle which mark the points of application of the unipolar electrode in the experiment in which figures 1 and 2 were obtained. *Br.*, bristles; *Nuc. dors. X*, dorsal vagus nucleus.

Fig. 5. Frontal section (rostral surface) through the medulla oblongata (cat) showing the position of the bristle which marks the point of application of the unipolar electrode in the experiment in which figures 1 and 2 were obtained. *Br*, bristle; *Nuc. dors. X*, dorsal vagus nucleus; *Tract. solitar*, tractus solitarius; *Nuc. XII*, nucleus of hypoglossal nerve; *N. X*, roots of vagus nerve; *N. XII*, roots of hypoglossal nerve.

Fig. 6. Tracing from x-ray film showing contour of the stomach (cat) before faradic stimulation of the cephalic half of the dorsal vagus nucleus.

Fig. 7. Tracing from x-ray film showing contour of stomach (cat) immediately after faradic stimulation of the cephalic half of the dorsal vagus nucleus. The dotted lines indicate the contour of the stomach immediately before the stimulus was applied. *p*, waves of contraction in the pylorus.

obtained, are shown in figure 4. It will be noted that the position is in the cephalic half of the dorsal vagus nucleus. The effects on the stomach of inserting a bristle into the dorsal vagus nucleus, in another experiment, are shown in figure 3.

Histological sections of the bulb, stained by the Weigert hemotoxylin method, show that the electrode was applied over the cephalic half of the dorsal vagus nucleus. Figure 5 shows the rostral surface of a frontal section through the dorsal vagus nuclei. The bristle marks the point at which the stimulation was applied with the unipolar electrode in securing the records shown in figures 1 and 2.

When the stomach is filled with a mixture of milk and barium sulphate and observed with a fluoroscope during and after stimulation of the dorsal vagus nuclei definite waves of contraction are observed passing over the pyloric portion of the stomach. The body of the stomach appears to diminish slightly in size. These changes persist for varying periods of time after the removal of the stimulus and during this time the contents of the stomach are rapidly passed into the small intestine. These facts are illustrated in figures 6 and 7. Figure 6 is a tracing from an x-ray film showing the contour of the stomach before stimulation of the nucleus and figure 7 is a tracing showing the contour of the stomach immediately after stimulation. The dotted lines in figure 7 represent the contour of the stomach before stimulation.

Discussion. Faradic stimulation over the cephalic half of the dorsal vagus nucleus of the cat, produces contractions in the stomach provided that the tonus in the stomach at the time of stimulation is of low degree. The contractions last for varying periods after the stimulus is removed. If the stomach is hypertonic at the time of stimulation the result is a diminution of tone.

The effects on the stomach of stimulation of the cephalic half of the dorsal vagus nucleus are similar to those obtained by M'Crea, M'Swiney and Stopford (1925) on faradisation of the peripheral ends of the cut vagi. These writers showed that, if the stomach is in a state of hypotonus at the time of stimulation movements are initiated, or if movements are already present they are increased. On the other hand, if the stomach is in a state of hypertonus at the time of stimulation there is a diminution of tone. The conclusions drawn by the majority of workers are that the vagus nerves carry both motor and inhibitory fibres to the musculature of the stomach. The presence of these fibres has been demonstrated by Langley (1898), Meltzer and Auer (1906) and Carlson (1922). Carlson (1922) showed that stimulation of the peripheral end of the vagus produces both inhibition and contraction in the cardia, depending on the tonic state of the cardia at the time of stimulation. Inhibition is more readily obtained if the cardia is in a state of hypertonus at the time of stimulation.

Molhant (1910) pointed out that the vagal motor fibres which supply the stomach have their origin in the cephalic part of the dorsal vagus nuclei. The present work shows that both motor and inhibitory effects are obtained in the stomach as a result of stimulating the cephalic half of the dorsal vagus nuclei. It therefore seems plausible to conclude that both the motor and inhibitory gastrovagal fibres have their origin in these nuclei. Furthermore it would seem that reflexes which influence the stomach in a motor way will have the dorsal vagus nuclei as components in their reflex arcs; also reflexes which produce inhibition of tone in the stomach, at certain periods, may pass through these nuclei.

SUMMARY

1. Faradic stimulation of the cephalic half of the dorsal vagus nuclei of the cat, causes contractions in the stomach; although if the organ is hypertonic at the time of stimulation inhibition of tone results.

2. Faradic stimulation of the cephalic half of the left dorsal vagus nucleus results in a more marked effect on the stomach than stimulation of the corresponding part of the right dorsal vagus nucleus.

3. Fluoroscopic examination of the stomach, during and after stimulation of the dorsal vagus nucleus, shows definite waves of contraction passing over the pyloric portion of the stomach. The body of the stomach also appears to be contracted.

I wish to express my appreciation to Prof. F. R. Miller for his helpful suggestions throughout this work.

The photography and histological work was executed by Mr. T. W. Stewart and the technical assistance was rendered by Mr. W. Byles.

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DETERMINATION OF THE SURFACE AREA OF THE WHITE RAT WITH ITS APPLICATION TO THE EXPRESSION OF METABOLIC RESULTS

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Whatever may be the theoretical significance of body surface as a biometric unit for the reference of metabolic rate measurements, it seems at the present time that the use of such a unit gives the least variable results for individuals differing greatly in size, and for different species. In this paper no discussion of surface area as related to heat production and dissipation by an organism will be entered into.

With the increasing use of the rat as a laboratory animal for metabolism experiments, there is a need for the determination of formulae for the estimation of surface area with an accuracy comparable to that attained in the measurement of heat production by present day methods and technic. Hitherto, surface area in the rat has been estimated by the use of the Meeh formula,

$$S = k W^{\frac{2}{3}}$$

The value of k was given by Rubner (1883, 1902) as 9.13 and by Pfaundler (1916) as 9.74. No statement has been found as to the number of animals or the methods used by these workers. Hill and Hill (1913) found the average value of k for five young rats of 50 to 129 grams weight to be 9.93. Carman and Mitchell (1926) measured the skin areas of 62 rats ranging from 25 to 461 grams in weight. They obtained an average constant of 11.36.

There are a number of objections to this formula. In it the assumption is made that body surface is a function of weight alone. A considerable increase in accuracy has attended the results of workers who have introduced a length or height measurement into the Meeh formula for other animals and man. Theoretically, such a measurement should be of aid in predicting the surface area of individuals who vary much from the pattern shape of the species, i.e., in obesity or emaciation. It would seem probable

that the use of such a measurement in the rat might increase the accuracy of prediction, even though casual observation indicates that changes from the pattern shape in rats are not as great as in many other species. Carman and Mitchell were not successful in attempts to increase the accuracy by introducing a function of body length. The measurement of length which they used, however, was from the withers to the root of the tail, expressed to the nearest half centimeter. A comparable length measurement has not, so far as we are aware, been used for other animals. We have made such measurements in all of the rats in the present series and find that they stand in no very constant relationship to total nose-anus length.

When metabolic rates are expressed with reference to body surface as predicted by the above formula with the constant 11.36 they amount to approximately 24 calories per hour per square meter for adult female rats. This value is more than 30 per cent lower than that for adult women or female dogs. It would seem that in rats under laboratory conditions, where body temperature is about the same as in man, the rate of heat production should be more nearly comparable.

This large discrepancy between the heat production of the rat and of man would seem to indicate that the value 11.36 for k is considerably too great. It is probable that this high value of k was due to the method of measurement used, which involved stretching the skin. Previously, in a few determinations of the skin area by the same method, one of us (L) obtained results in close agreement with those of Carman and Mitchell. The average constant in the Meeh formula, for six adult rats was 11.49.[1] It was observed at that time that the area of the skin could vary within wide limits, depending upon the amount of stretching. The skins of rats of all ages are remarkably loose and apparently somewhat elastic. Certainly the true body surface is not the total area of the stretched skin.

These considerations, together with the fact that in some work on metabolism under way in this laboratory a more accurate basis of comparison of the metabolic rate of the rat with that of man and of other animals was desired, have led us to undertake the task of measuring the skin areas of a large series of rats by a method which eliminates errors due to stretching.

A number of methods were considered for obtaining true body surface areas without stretching the skin. The small size of the rat makes difficult the application of the paper mold or plaster cast methods which have given satisfactory results for other species in the hands of a number of workers. The method finally adopted was to dip the animal in a quickly drying, nonshrinking lacquer which was allowed to harden before the animal was skinned. Such a varnish after hardening is still flexible, but does not

[1] The area of the ears was included in the measurements.

stretch.[2] It is believed that the accuracy of this method is greater than that of paper molds or plaster casts. The actual area given is that of the outside surface of the skin, since the outline is traced along the cut edge of the hardened varnish. The skin after removal is a perfect mold of the body. Any small wrinkles are preserved just as they were on the animal. An idea of the exactness of the molding of the body is given by the fact that it was necessary to cut the skin into from ten to fifteen pieces in order to make it lie flat upon the paper. The unvarnished skin may be stretched

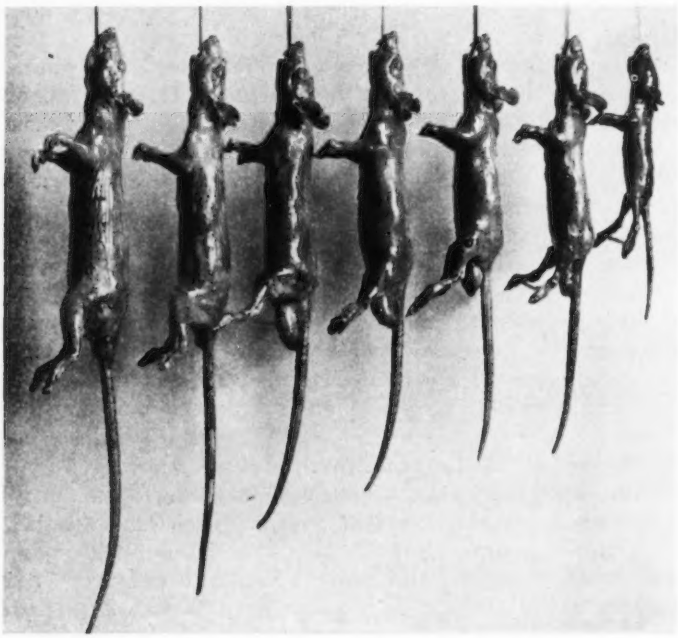

Fig. 1

flat in a single piece. Figure 1 is a photograph of several varnished rats showing the mold of the body secured, the method of spreading the legs and the position of the animal while the coating was drying. The method has been used on monkeys (Macacus rhesus) weighing as much as six kilograms, with excellent results. Even for so large an animal a single heavy coat of varnish is sufficient.

[2] Two commercial nitro-cellulose enamels, "Duco" and "Nitro-Valspar" were used. The latter preparation gave the better results.

Since rats are ordinarily starved for 18 to 24 hours before a metabolism determination, it was thought desirable to starve them similarily before making body surface measurements. The rat was then killed and body weight and the several length measurements were recorded immediately. Five linear measurements were made, nose-anus length, withers-anus length, length from nose to end of tail, reach of the forelegs and stretch of the hind legs. The hair was removed at first by clipping and later by dipping for a few minutes into a solution of barium sulphide. The latter method was more satisfactory since the hair on the head and around the legs was taken off. The rat was then washed, and dried or sponged with alcohol. It is necessary that the skin be dry for the varnish to adhere tightly. The animal was then dipped into the nitro-cellulose lacquer, suspended by the teeth, and the excess lacquer allowed to drain off. The legs were held away from the body by sticks between the paws. The body was hung in a current of warm air and ordinarily the varnish became hard within an hour. The rat was then skinned, the pelt cut into as many pieces as necessary, and the pieces pinned out flat on paper. The areas were outlined with pencil, traced on smooth paper and measured with a compensating polar planimeter. The toes were allowed to stick together while the varnish hardened, and the whole foot was split parallel with the palm. This gives both surfaces of the entire foot but does not give quite all of the area on the side of each toe. The error involved, however, is very small. The skin of the tail was removed and measured. The ears were cut off around their bases, pinned flat and the outline traced. The area of each ear was multiplied by two to give both surfaces. Carman and Mitchell did not include the areas of the ears in their measurements. These surfaces in small rats may amount to a maximum of about 5 per cent of the total.

The accuracy of the method was checked in a number of ways. It was thought that the varnish might contract in drying and thus tend to decrease the skin areas. This was tested by using a gold-beater's skin balloon attached to a water manometer. A pressure of a few centimeters of water was placed on the membrane to keep it distended. It was then dipped into the varnish and allowed to dry. After correcting for changes in temperature it was found that no measurable contraction had occurred.

Two outlines of one skin were made by two people. The difference between the measurements was 0.8 per cent. The difference between the measurements made by one person of two outlines of each of four skins was 0.03, 0.1, 0.06 and 0.4 per cent. Measurements by two people of the same outlines differed by 0.01 and 0.03 per cent. All areas were measured at least twice.

Seventy-two rats, 27 females and 45 males, ranging from 19 to 418 grams in weight were used in the series. Animals in all conditions of health

TABLE 1

SERIAL NUMBER AND SEX	AGE AND CONDITION	BODY WEIGHT	LENGTH FROM NOSE TO ANUS	SKIN AREA MEASURED	WEIGHT/LENGTH	SERIAL NUMBER AND SEX	AGE AND CONDITION	BODY WEIGHT	LENGTH FROM NOSE TO ANUS	SKIN AREA MEASURED	WEIGHT/LENGTH
	days	*grams*	*cm.*	*sq. cm.*			*days*	*grams*	*cm.*	*sq. cm.*	
1f	18A	19	9.5	73	0.280	37f	C	174	20.0	262	0.279
2m	21B	21	9.9	76	0.278	38f	C	176	20.6	305	0.272
3f	30B	32	11.5	103	0.276	39f	C	183	21.3	279	0.267
4m	32B	34	11.8	105	0.275	40f	185B	191	20.7	269	0.278
5f	40A	46	12.7	123	0.282	41m	C	192	21.9	317	0.263
6m	45B	48	13.4	135	0.271	42f	C	199	20.8	309	0.281
7f	50A	63	14.3	147	0.278	43m	C	212	21.4	300	0.279
8m	55A	63	13.6	152	0.293	44f	B	216	21.2	321	0.283
9m	60C	65	14.5	154	0.277	45m	C	218	22.2	335	0.271
10m	57A	68	14.6	156	0.280	46m‡	C	219	22.3	310	0.270
11m	64C	72	16.2	182	0.257	47m	C	221	21.6	336	0.280
12f	62B	74	14.5	165	0.289	48m	B	222	21.5	316	0.282
13m	64C	79	16.3	174	0.263	49m	C	228	22.9	327	0.267
14m	63A	79	14.8	169	0.290	50m‡	B	231	22.2	328	0.276
15m	66C	83	16.1	190	0.271	51m	C	238	22.3	349	0.278
16f	72B	86	15.6	181	0.283	52m	B	243	22.3	349	0.280
17f	72C	87	16.8	181	0.264	53m	C	245	22.8	330	0.274
18m	73A	88	15.1	183	0.295	54m	C	250	22.6	349	0.279
19f	73B	88	16.0	174	0.279	55m	C	254	22.8	367	0.278
20m	85B	106	17.0	209	0.279	56m	A	257	22.6	335	0.281
21m	86A	118	17.5	224	0.280	57m	A	261	21.5	340	0.297
22f	105D	118	19.2	238	0.255	58m	B	263	22.9	342	0.280
23f	95B	120	17.6	206	0.280	59m	B	269	22.2	351	0.291
24f	95B	130	18.2	242	0.278	60m	A	271	21.2	355	0.305
25f	90A	133	17.8	229	0.287	61m	B	273	22.7	342	0.286
26m	95B	136	16.7	223	0.308	62m	B	275	22.4	343	0.290
27f	C	140	19.8	263	0.262	63f§	B	276	22.4	364	0.291
28f	122B	151	19.6	247	0.272	64m	C	290	23.9	404	0.277
29m	D	152	19.7	268	0.271	65m	B	291	23.4	363	0.283
30f	A	153	18.8	232	0.285	66m‡	B	320	22.8	384	0.300
31f	B	156	19.1	252	0.282	67m	B	341	24.6	419	0.284
32m	C	156	20.1	256	0.268	68m	A	365	24.0	433	0.298
33f	D	163	20.5	261	0.267	69m	B	366	25.9	450	0.276
34f	B	169	18.6	263	0.297	70m	B*	384	25.6	461	0.284
35f	125A	171	19.6	250	0.283	71m	A	388	24.9	447	0.293
36f	C	172	20.5	298	0.271	72m	A†	418	25.2	461	0.297

* 24 grams of water given by colon.

† 32 grams of water given.

‡ Castrated two months previously.

§ 13th day of pregnancy.

were purposely selected. Some had much body fat, others were much emaciated, one female was pregnant, and three males had been castrated. It was desired to emphasize as much as possible differences in the pattern shape in order to determine a formula for body surface which would serve for under- and over-nourished animals as well as for normal ones.

In table 1 the observed condition of the rat is indicated by the letters A, B, C, or D. By this scale A represents what was considered an excellent condition, with considerable body fat and a heavy sleek coat of hair. The letter D designates rats considerably emaciated. The letters B and C designate more or less arbitrarily, grades between these extremes. The condition indicated by the letters does not in all cases agree with that indicated by the nutritive ratio, which is probably a better index of bodily condition. Marked obesity is seldom found in rats and no very obese animals were available to us. In two rats attempts were made to simulate obesity by the administration of water. The animals were anesthetized with amytal. The water was given slowly by way of the colon and sufficient time allowed for much of it to be absorbed. Since the body weight was recorded only to the nearest gram, differences in the specific gravity of body fat and water would introduce no error.

Results. The data of the skin area measurements are given in table 1. When these data were applied in a formula of the Meeh-Rubner type

$$S = k W^{\frac{2}{3}} \tag{1}$$

the average value of k was found to be 9.00 ± 0.05, with a standard deviation of ± 0.604 and a coefficient of variation of 6.71 per cent. This constant is 21 per cent less than the value 11.36 as determined by Carman and Mitchell for the same formula and is in fairly close agreement with the original value of 9.13 determined by Rubner. For the same range of weight (50 to 130 grams) the values of k also agree well with those given by Hill and Hill (1913) It was found, however, that there was a progressive decrease in the individual values of the constant with increasing size and weight. The deviation from the average ranged from about +14 per cent in the small rats to about −9 per cent in the large ones. Of the first 36 animals listed in the table, 26 had constants greater than the average k and of the remaining 36 animals 28 had constants with minus deviations. Carman and Mitchell found no such change in the values of k for the rats in their series. The probable explanation is that the skins of the smaller and younger rats stretch more than do those of larger and older ones.

This progressive decrease in the constant suggested that the value of the exponent of W was too great. Accordingly, the exponent of W was varied from 0.40 to 0.80 and the coefficients of variation of the corresponding average values of k were determined. These coefficients of variation with the several exponents were as follows: 0.40—13.4 per cent; 0.50—

7.9 per cent; 0.59—4.67 per cent; *0.60—4.55 per cent;* 0.61—4.78 per cent; 0.667—6.71 per cent; 0.70—8.5 per cent;0.80—15.5 per cent. It is apparent from these data that the best value for the exponent of W in the simple Meeh formula for the rat is very nearly 0.60. Cowgill and Drakbin (1927) showed, in the same way, that with Carman and Mitchell's data the best value of the exponent was 0.667. It is believed that the difference in the results on the rat is due to the inaccuracies involved when the skin is stretched.

The data were then applied in formula (2), of the Meeh-Rubner type,

$$S = k\,W^{0.60} \tag{2}$$

with the value 0.60 for the exponent of W. In this formula the average value of k was 12.54 $\pm$0.05. The agreement was considerably closer than with formula (1). The average deviation was $\pm$3.61 per cent of the mean; the standard deviation was $\pm$0.570 and the coefficient of variation was 4.55 per cent of the mean. Seventy-two per cent of the individual values of the constant had a deviation of not more than 5 per cent from 12.54 and 27 per cent had a deviation of less than 10 per cent. The greatest deviation was 11.5 per cent.

The agreement of calculated and measured surfaces was also much better by this formula. By formula (1) the average deviation of the calculated from the measured areas was $\pm$6.4 per cent; by formula (2) the deviation was $\pm$3.6 per cent. With formula (2) there was further no consistent variation of the individual constants with increasing size. For the animals in a poor nutritional condition, however, the accuracy was not as great as for those in good condition.

Attempts were made with several formulae to increase the accuracy by introducing a function of length. One such expression used, namely,

$$S = k\,W^{0.425}\,L^{0.725} \tag{3}$$

is the same as the Du Bois height weight formula for human beings, except for the vaue of k, and is also similar in principle to the *metabolic index* which Gulick (1925) has used for rats. This formula is perfectly bi-dimensional in character. For our series of rats the average value of K was 3.57 $\pm$0.02, with a coefficient of variation of 6.25 per cent. The accuracy of prediction is thus not as great as with formula (2) and only slightly better than with formula (1). In other trials, the values of the exponents of W and L were changed, both with and without regard for keeping the formula perfectly bi-dimensional. None of these attempts gave results as accurate as were obtained with formula (2). Linear measurements other than total body length were not found to be of value in constructing more accurate formulae.

Cowgill and Drabkin found that the use of a "*nutritive correction factor,*"

similar in principle to von Pirquet's *gelidusi*, gave the best formula for estimating the body surface of dogs. Von Pirquet (1917) showed that the ratio of the cube root of ten times the weight to the sitting height, gave a reliable index of the nutritional state of children. Cowgill and Drabkin used for their nutritional correction factor the ratio $\frac{W^{\frac{1}{3}}}{L}$. They determined the ratio for the most obese dog obtainable and used this value as a standard with which to compare other values of the ratio. We have used the same principle in the determination of a similar formula for the rat, and find that a somewhat greater accuracy is attained than with any other formula we have used. The values of $\frac{W^{\frac{1}{3}}}{L}$ for each rat are given in table 1. We have retained the terms used by Cowgill and Drabkin, of N_m for the standard or maximal and N_{obs} for the individual nutritive indices. The highest ratio obtained was 0.308. The value 0.310 was selected as the arbitrary standard.

Formula (4) which contains the nutritional correction factor, and which gave the closest agreement with the data, is as follows:

$$S = k\, W^{0.61} \times \frac{0.310}{N_{obs}} \tag{4}$$

In this formua the average value of k was 10.76 $\pm$0.04, with a standard deviation of $\pm$0.444 and a coefficient of variation of 4.12 per cent. Seventy-four per cent of the individual values of k had a deviation of less than five per cent from 10.76 and 26 per cent had a deviation of less than nine per cent. The greatest single deviation was $-$8.9 per cent. The exponent 0.61 for W gave slightly more consistent values of k in this formula than 0.60. The agreement of calculated and measured surfaces was also slightly better. With the exponent 0.60 the average constant was 11.31, with a standard deviation of $\pm$0.471 and a coefficient of variation of 4.17 per cent.

The increase in accuracy by the use of such a formula involving a function of length is not as great as that found by Cowgill and Drabkin for the dog. However, as pointed out above, differences in the pattern shape are not nearly so marked in the rat as in the dog. The use of formula (4) is recommended for rats that are emaciated, markedly obese or that have shown abnormal growth.

For the estimation of surface area in adult rats in good condition, formula (2) may be preferable because of its simplicity and the fact that no length measurements are necessary. This is sometimes a decided advantage. In our experience, the only satsifactory method of obtaining body length measurements on living rats is to use light anesthesia. The animals may then be placed back downwards on a strip of paper, the head held by a

wire loop over the teeth, the body straightened by a slight traction on the tail, and marks made opposite the anus and the tip of the nose. Repeated measurements made in this way check within 2 mm.

It is apparent that for both formulae (2) and (4) there is no significant variation in the values of k with age or size. For both the simple formula and that involving the nutritional correction factor, the average of the constants for the 36 rats weighing less than 172 grams is almost identical with the average of the constants for the 36 weighing more than 173 grams. In both formulae the value of k is slightly greater for males than for females. In formula (2) the average for males is 12.62 and for females 12.44. In formula (4) the average for males is 10.87 and for females 10.62. This difference is probably due to the additional area of the scrotum in males, which may amount to upwards of 8 or 9 square centimeters. As is apparent from figure 1, this area is considerably greater than the increase in body surface that would be caused if flesh equal in weight to the scrotum and its contents were evenly distributed over the animal. In the three castrated. males the average constant is slightly less than the average for the females. The most of the rats in poor condition are concentrated in the middle of the weight range. The average deviation of the constant is slightly greater for the animals designated C or D than for those designated A or B, but there is no consistent variation in one direction.

The final test of the usefulness in metabolic work of a formula for the estimation of body surface area is the agreement of results of basal metabolic rate determinations in animals of widely different weights and various species pattern shapes. As Harris and Benedict (1919) have pointed out, the general applicability of the so-called "surface area law" of Rubner cannot be said to have been rigorously tested because of the inaccuracies of the formulae hitherto used for the estimation of body surface in different species. Since the accuracy of formulae (2) and (4) of this paper is comparable to that of formulae for the dog and man, a valid comparison of the basal heat production in the rat with these species may be made. Mitchell and Carman (1926) published data of basal metabolic rate measurements on rats ranging from 84 to 251 grams in weight. Using their formula for surface area, they found the average basal heat production in starved female rats to be 571 $\pm$7 calories per day per square meter of body surface, with a coefficient of variation of 8.0 per cent. In male rats the corresponding heat production was 600 $\pm$11 calories, with a coefficient of variation of 13.5 per cent. We have calculated from their data the daily heat production per square meter of body surface as predicted by our simple formula (2). For the female rats the rate is 730 $\pm$8 calories, with a coefficient of variation of 7.6 per cent. For the male rats the rate is 777 $\pm$14 calories, with a coefficient of variation of 13.2 per cent. The agreement of the metabolic results, as shown by the coefficients of variation, is slightly better with our formula than with the Meeh-Rubner

expression. For a more conclusive test, rats of more extreme weight ranges should be used.

Just as significant as the better agreement of metabolic results in the rat, is the fact that, while the heat production of the rat is brought into much closer agreement with that of the dog and of man, there is still a considerable and apparently valid difference. Cowgill and Drabkin showed that with body surface predicted by their formula, the heat production of adult female dogs averaged 836 calories per day per square meter, or about two per cent less than the heat production in women. With Carman and Mitchell's formula for surface area, the heat production in the female rat is 33 per cent less than that in women, and in the male rat 35 per cent less than in men. With our formula 2 the differences are 14 and 16 per cent less respectively. These differences are rather large, and it would appear that there is a discrepancy between the heat production of the rat and of man, expressed with reference to body surface area, which is somewhat greater than can be attributed to errors in the estimation of either body surface or metabolic rate.

SUMMARY

The skin areas of 72 rats, ranging from 19 to 418 grams in weight and in various conditions of nutrition, were measured by a method which eliminates errors due to stretching. The animals were given a coating of a quickly drying varnish before skinning. Formulae for the prediction of body surface were constructed on the basis of the data obtained. Two formulae were selected which gave the best agreement with the data. The simpler but slightly less accurate one is a modified Meeh-Rubner formula, in which the exponent of W is 0.60 and the constant 12.54. The best formula found involves the use of a correction factor for the nutritional state. The heat production of the rat expressed with reference to body surface, as predicted by these formulae, is brought into closer agreement than hitherto with the heat production of human beings.

We are indebted to Mr. Philip Solomon for assistance in the large number of calculations involved in this work.

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THE MOTOR AND SECRETORY ACTIVITY OF THE STOMACH DURING ACUTE AND CHRONIC OBSTRUCTIVE JAUNDICE IN DOGS

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The present investigation is an attempt to segregate the possible rôle of impaired gastric functions in the symptom complex of jaundice. The experimental data touching this problem are meagre, and clinical data are either too fragmentary or insufficiently controlled to permit conclusions.

LITERATURE. Von Jasch (1) found diminished HCl in certain cases of catarrhal jaundice. Von Noorden (2) reported normal HCl in three cases of cholelithiasis and two catarrhal jaundice cases. Riegel's (2) findings are similar to Von Noorden's. Leva (3) noted achylia in patients with gall stones, and stated that the HCl reappeared only after the icterus had subsided. Hohlweg (4) found hypochlorhydria in studying the effect of cholecystectomy upon gastric secretion in man and dogs.

Myer, Ivy and McEnery (5), studying gastric secretion on four cholecystectomized dogs, found no depression of gastric secretion, and three out of four dogs showed a variable degree of hypersecretion. These workers also found that 50 cc. of bile by stomach tube caused increased secretion of gastric juice, thus confirming Sokolov (6).

Rydgaard (7), Rohde (8), Glaser (9), Dangschat (10), Wohl (11), Bonar (12), Gatewood (13), Rost (14), and others, have reported achlorhydria in cases of gall stones.

Hayem (15) gives clinical proof of hypersecretion and hyperchlorhydria accompanying cirrhosis of the liver, but he did not find this condition of the stomach always accompanying jaundice.

Simnitzky (16) studied twelve cases of jaundice, the majority of which were catarrhal jaundice, in which he found gastric hyperacidity and hypersecretion. Following this clinical study he studied gastric secretion on three gastrostomy dogs, and two Pavlov pouch dogs before and after producing obstructive jaundice. In these animals he found gastric hyperacidity, the free acid being the chief factor. The work of Griffiths (17) confirms Simnitzky. Hunter (18) studied eight cases of obstructive jaundice in man. His findings are not uniform, but agree in the fact that the maximum acidity is remarkably high, the average being free HCl 65,

and total HCl 78 units. Four cases show copious transparent fluid with high acidity of the fasting stomach content, an abrupt rise and a marked plateau. Emptying was usually delayed. Estimating the obstruction by the nature of the lesions and its intensity of jaundice, it was found that the highest acidity coincides with the greatest obstruction.

EXPERIMENTS ON GASTRIC MOTILITY. Gastrostomies were made in healthy dogs. The dogs were fed a stock diet consisting of meat, 200 grams; bread, 200 grams; milk, 150 cc.; bone meal.

Three to five days after the operation gastric motility was recorded by the balloon method using a bromoform manometer. The tests were run eighteen hours after the previous meal. The tests were run for approximately 180 minutes in all cases. It was felt that under these conditions average figures would represent the average motility of the empty stomach as effected by jaundice.

Control experiments were made to determine the average motor activity of the stomach before jaundice. These controls included intravenous injections of 0.9 per cent sodium chloride solution during the first period of high gastric contractions, to determine if the injections per se influence the gastric motility. The salt solution injections did not modify the hunger contractions. The experiments with the intravenous injection of bile were alternated with control experiments. The bile was injected in quantities from $\frac{1}{8}$ to $\frac{1}{2}$ cc. per kgm. of body weight. The desired quantity of bile was diluted with 0.9 per cent sodium chloride solution, to 10 cc., and was warmed to 40°C. before injection.

PREPARATION OF BILE FOR INTRAVENOUS INJECTION Dog bile was evaporated to dryness on the water bath and finally dried in a desiccator. The dried product was reduced to fine powder. Note was taken of the volume of the original bile represented by 1 gram of the dried product. When ready for use the dry powder was dissolved in the required volume of warm water, immersed in a beaker of boiling water for 15 minutes and then filtered. In this way a standard bile product was always available. Samples of the same preparation were used throughout this work.

After concluding the injection experiments chronic jaundice was produced by double ligation and section of the common bile duct and the observation on gastric motility continued.

The following precautions were observed in handling the animals:

1. The animals were thoroughly trained to lie on the table without any restraining for periods in excess of three hours, before any experimentation was attempted.
2. Food, exercise, experiments, etc., came at the same time each day.
3. The animals were treated kindly but firmly. At no time were the animals afraid of or excited by the presence of the experimenter.

4 The animals were bathed regularly and kept free from mange and fleas.

The data are given from four of the animals used in the motility experiments. Dog 1 lived for 206 days after the ligation of the common bile duct. Dog 2 died just after the operation. Dogs 3 and 4 lived 39 and 51 days respectively. A full description with complete data from dog 1 is presented, the other animals are only summarized in graphic form. This

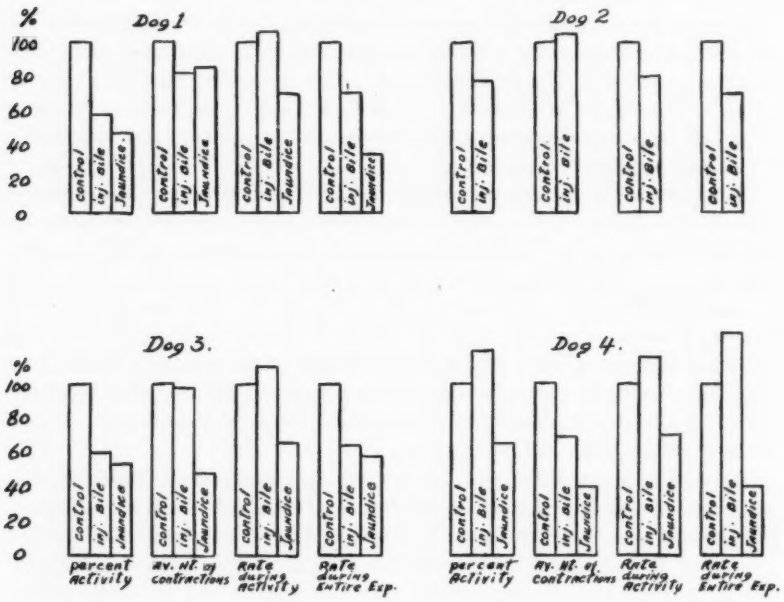

Fig. 1. Showing the effect of intravenous injection of bile and of chronic jaundice on the motor activity of the stomach.

is done because the effect observed following the several procedures was essentially the same for all the dogs.

Dog 1. This dog (no. 1, male) prior to the production of jaundice was very active, alert and in splendid condition. His weight during the control experiments was about 10 kgm., pulse 76, and rectal temperature 100. Intravenous bile injections caused a decrease in the per cent of activity and the height of the hunger contractions per minute. In one case there was vomiting following the bile injection, and at the conclusion of that experiment he ate only one-third of his usual meal. On all other periods he ate all of his food after bile injection. His appetite was voracious

throughout the control periods and also throughout the period of temporary jaundice (with the above exception). There were periods of diarrhea during the temporary jaundice experiments which necessitated the discontinuance of the experiment for several days at various times. Muscle twitchings, retchings, and general malaise was often noted for short periods following the bile injections.

On October 29th the dog was made permanently jaundiced through double ligation and section of the common duct. His weight at that time was 10.5 kgm., pulse 72, and rectal temperature 100. For three weeks following the operation there was depression and little desire for food. In all the animals there was a diminished appetite and in dog 4 (motility) there was almost a complete anorexia for days at a time. There was for

Fig. 2

Fig. 3

Fig. 2. Showing the edema of the hind legs and feet in dog 1 on the 196th day of obstructive jaundice.

Fig. 3. The gall bladder of dog 1 on the 206th day of obstructive jaundice, showing the organized cast.

seven weeks a steady decrease in body weight. There was also a tendency toward bradycardia. Then there occurred a remission, and a general increase in weight, and an increased pulse rate. From this time on there were exacerbations and remissions of the symptoms. Ascites developed, and on February 28th, March 8th and May 3rd paracentheses were done. The ascites probably accounted for the animal's gain in weight. At each paracenthesis more than two liters of a greenish transparent fluid was collected. The animal demonstrated all the usual symptoms of jaundice. The skin and mucous membrane of the eyes and mouth were deeply stained, the urine was highly colored, polydipsia, pruritus, cachexia, edema, markedly of the hind legs and feet, and an atrophy of the shoulder muscles. Figure 2 shows the edematous condition of the animal. The animal died May 21st, having lived 206 days with jaundice, which is considerably

TABLE 1

Showing the data on the motor activity of the empty stomach collected from dog 1

Each entry for the period after the production of chronic jaundice represents the average figures for three experiments.

DATE	EXPERIMENTAL CONDITIONS	ACTIVITY	AVERAGE HEIGHT OF CONTRACTIONS	NUMBER OF CONTRACTIONS PER MINUTE DURING PERIOD ACTIVITY	NUMBER OF CONTRACTIONS PER MINUTE DURING PERIOD EXPERIMENT	HIGHEST TONUS DURING EXPERIMENT	AVERAGE TONUS DURING EXPERIMENT
		per cent	*mm.*			*mm.*	*mm.*
September 3	Control	100	168	1.24	1.24	20	Low
September 5	Salt injection	90	177	0.75	0.67	40	Near base
September 7	Salt injection	93	174	0.79	0.64	Low	Base
September 8	0.25 cc. bile	30.6	78	0.50	0.15	Low	
September 10	0.125 cc. bile	100	200	1.18	1.18	20	5
September 11	Control	100	200	0.75	0.75	60	High
September 12	0.4 cc. bile	86.4	115	0.50	0.42	80	60
September 13	Control	88	172	0.72	0.63	14	Low
September 14	0.5 cc. bile	69.8	166	0.50	0.32	+30	Base—30
September 15	Control	68	196	0.40	0.27	20	15
September 17	0.5 cc. bile	80	187	1.62	1.31	45	20–30
September 18	Control	100	200	0.80	0.80	18	Base—18
October 4	0.5 cc. bile	9	200	1.00	0.10	70	10—70
October 25	0.5 cc. bile	11	102	0.40	0.07	10	−10
October 29	Operated—common bile duct cut						
	Chronic jaundice						
November 5–9		43	109.0	0.49	0.31	30.0	Near base
November 11–15		36.4	140.0	0.35	0.19	35.0	Base—20
November 21–28		67.3	216.0	0.52	0.35	70.0	Base—70
November 30–December 9		56	94.0	0.31	0.25	42.0	Base—42
December 12–19		46.6	215.0	0.47	0.21	40.0	Base—30
December 24–30		47.2	148.0	0.47	0.22	16.0	8–10
January 5–16		26.7	116.0	1.06	0.31	48.0	Base—14
January 19–23		33.8	103.0	0.36	0.18	20.0	Near base
January 25–30		62.1	125.0	0.56	0.34	48.0	Mostly at base
February 1–7		33.9	85.0	0.86	0.28	16.0	8–12
February 9–23		57	147.0	0.45	0.31	12.0	Base
March 5–26		62.6	139.0	0.77	0.43	20.0	Base
April 2–26		63.4	106.0	0.83	0.53	Base	Base
May 10–11		0	0	0	0	28	Base
May 21	Dog died						

February 28—Paracenthesis.
March 8—Paracenthesis.
May 3—Paracenthesis.

longer than any other animal reported (so far as I am able to find, about three months longer).

On autopsy the following was noted: The mucous membranes of the peritoneum, the periosteum, the meninges, and the blood vessels were deeply stained. There was a general atrophy of the skeletal muscles, a generalized emaciation (disappearance of fat), and the fat which was present was bile stained. The heart was dilated. The liver was hard, its borders rounded, and of a slightly mottled appearance. Sections of the liver showed fatty changes, but there was no evidence of cirrhosis in so far as there was no increase in the intralobular connective tissue, though there seemed to be an enlargement of the hepatic cells. The ductus choledochus had not established communication with the duodenum. The gall bladder and lower portion of the duct system was filled with a firmly organized cast. (Fig. 3 shows the organized cast in the gall bladder.) This cast was of a pale waxy color. Chemical analysis showed the presence of calcium, cholesterol and protein. Mucin was not detectable. This animal had developed white bile and presents an interesting finding, namely, that the formation of the organized cast of the lower ducts and gall bladder is very uncommon. This was the only dog to show an organized cast in the gall bladder, but two of the others had "white bile." The peritoneal cavity contained about 500 cc. of transparent ascitic fluid (this was the only animal to have a transparent ascitic fluid). The kidneys showed the usual nephritis which accompanies prolonged jaundice.

On examination of table 1 (which contains most of the data collected from the experiment on dog 1) and figure 1 one will observe the following:

1. The average time of gastric motility was in every case longer during the control experiments than during chronic jaundice. In three animals out of four the time of activity was shortened by temporary jaundice as compared with the control experiments. Also the height of the contractions as well as the rate of the contractions was diminished by the intravenous injection of bile.

The mechanism of the motor depression is not known, however, a priori it would seem that at least two factors are involved. That bile constituents, especially the cholic acid fraction, are extremely toxic is well known. Since the muscles are bathed with blood containing abnormal concentrations of these substances, probably the depression is partly due to a direct action of bile upon them. The depression may be due to a nervous inhibition. This is in accord particularly with the views of Hayem, Simnitzky, and Hunter. These workers found obstructive jaundice to cause hyperacidity of the fasting stomach. This factor, if present, might explain the depression of gastric motility, since Carlson (19) has shown that acid in the stomach diminishes hunger contractions.

The depression due to temporary jaundice (bile injection) is of short

duration; usually the hunger mechanism was functioning normally before the end of the experiment.

The immediate effect of injecting bile was inhibition of the hunger contractions and an abrupt fall in the gastric tonus. The fall in the tonus was fleeting for the tonus as well as the hunger contractions recovered to the pre-injection level within the period of the experiment.

The average tonus was lowered by the production of chronic obstructive jaundice. The tonus waves were of normal height but the time between the periods of increased tonus was lengthened.

SECRETION. Pavlov pouches were made in healthy dogs. The dogs were fed the same stock diet as in the motility experiments. Records of gastric secretion were started seven to ten days after the operation. The continuous secretion was collected for a $\frac{1}{2}$ hour period. Then 100 grams of hamburg was given as a test meal. Collections were made at half hour intervals for two hours following the test meal. The free and total acidity was determined. After a series of control experiments several experiments were done in which 0.9 per cent sodium chloride was injected intravenously at the end of the first one-half hour period of continuous secretion. These injections had no demonstrable effect upon the gastric secretion. The bile was injected, as described under the motility experiments, at the end of the first one-half hour of the continuous secretion. The test meal was given an hour later.

All the experiments on gastric secretion were carried out as described except that in dogs 3, 4, 5 and 6 in the series on temporary jaundice, the test meal was given immediately after the bile injection. One milligram histamine was injected in place of the test meal in dogs 3 and 6.

Following the bile injection experiments, chronic jaundice was produced by double ligation and section of the common duct, and the observation on gastric secretion continued. Dogs 1, 2, 3 and 4 lived 61, 38, 34, and 19 days respectively. Dog 6 had to be sacrificed due to prolapse of the pouch. Dog 5 died with bile peritonitis following the ligation and sectioning of the common duct. It was found that if the ligatures were placed too near the duodenum there is an insufficient blood supply to the duct, and necrosis occurred at the point of the ligature.

RESULTS. An examination of figure 4 shows the average rate of secretion of each dog during the control periods, the periods of temporary jaundice, and the periods of chronic jaundice. It will be observed that in dogs 1 and 2, in which the bile was injected at the end of the first half hour of continuous secretion, the rate of continuous secretion was diminished by the injection of bile, and the feeding of the test meal at the end of the third half hour did not induce increased secretion. In these animals during the period of chronic jaundice the test meal given at the end

of the first half hour continuous secretion induced less secretion than the controls.

The total and the free acidity of the gastric juice was greatly diminished by the injection of bile. Following the injection of bile the test meal did not cause the usual rise in acidity observed under the control conditions. In neither of these animals, no. 1 and no. 2, did the test meal increase the acidity of the juice over that of the continuous secretion level. This was true for both total acidity and free acidity. After the production of

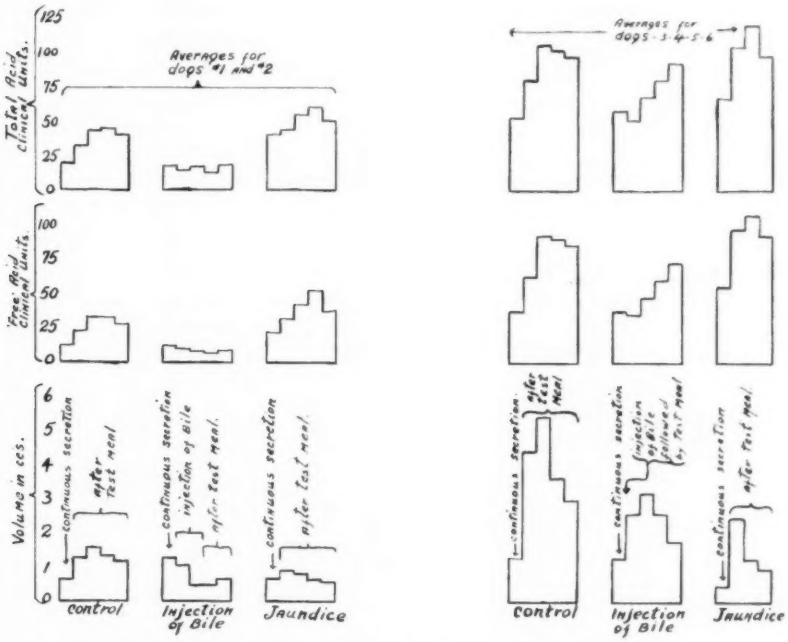

Fig. 4. Showing the effect of intravenous injection of bile and of chronic jaundice on gastric secretion.

chronic jaundice, these animals gave a continuous secretion of high acidity, particularly the free acid fraction, and responded to the test meal by secreting a juice of higher than normal acid values for that particular dog. Although the values were higher than normal the secretion curve was of normal contour.

In dogs 3, 4, 5 and 6 the test meal was given immediately following the bile injection at the end of the first half hour of continuous secretion. In these animals the test meal increased the rate of secretion over the continuous rate, but less than in the control experiments.

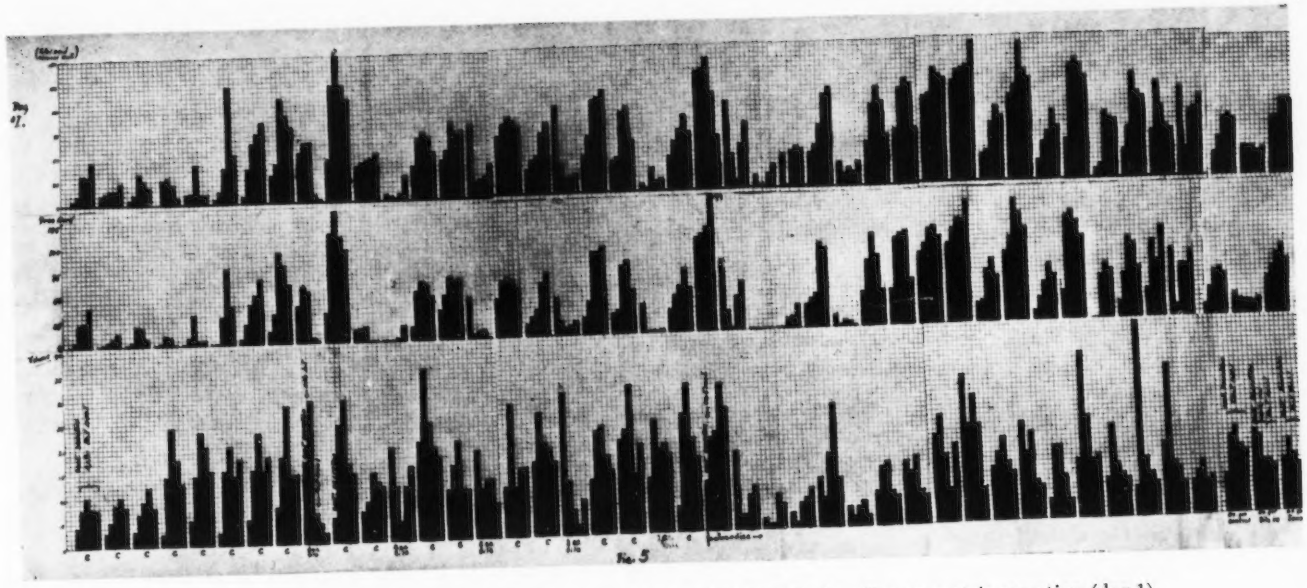

Fig. 5. Showing the effect of intravenous injection of bile and of chronic jaundice on gastric secretion (dog 1).

The free and total acidity was also lower. The immediate effect of the injection of bile was to greatly lower the gastric acidity. Only in one animal did the average figure show a return to control acidity values during the two hours following the injection. During the chronic jaundice experiments, the average acidity of the continuous secretion was increased over the control values, and the effect of the test meal was to augment the acid secretion considerably above the control values. A tendency was noted indicating a retardation in the time of development of the maximum acidity.

Figure 5 shows complete data on dog 1 (secretion). The findings from the other animals are summarized only (fig. 4), since the results were similar to those in figure 5.

A review of all the data collected from these experiments seems to indicate that the production of experimental obstructive jaundice in dogs induces the following changes in gastric function:

1. The rate of the hunger contraction is diminished.
2. The periods of activity (hunger) are shortened.
3. The magnitude of the individual hunger contractions is diminished.
4. The volume of gastric juice secreted is diminished.
5. The concentration of acid in the gastric juice is increased.

Discussion. The sudden exposure of the tissues to the toxic factor or factors in bile is a condition probably found only in cases of rupture of the gall bladder. This sudden bile intoxication induces in the dogs a series of acute disturbances such as a general depression, marked twitchings of the skeletal muscles, retching, occasional vomiting, increased thirst, diarrhea, and anorexia.

The mechanism by which the chronic jaundice brings about the manifested derangements in the gastric hunger mechanism is at present largely a matter of conjecture. The hepatic system, an outgrowth of the gut, may give origin to reflex nervous stimuli responsible for the observed alterations in gastric motility and secretion. One cannot say whether these stimuli are the result of increased intrahepatic pressure or of the increased cystic pressure.

In the cases observed where there was white bile or an organized cast the gall bladder and the lower ducts seemed greatly shrunken. In the other animals there was great distention. Since consistent results were obtained in all the animals it seems that this pressure factor is not of great importance. It is possible that the mere mechanical stimulation from the cast may give rise to the nervous reflexes.

Cholemia may be an important factor. If the toxic substances depressing the gastric functions were the sole cause of the depression, the hunger contractions should progressively decline, provided cholemia steadily increases throughout the period of jaundice. But the degree of cholemia

at the different stages of obstructive jaundice is not known and even if the cholemia were progressive the gradual emaciation of the dogs during jaundice may partly overcome the inhibition from cholemia.

The factors which are known to govern the acidity of gastric juice are 1, the chlorides of the blood, 2, the secretion rate. MacLean and Griffiths (20) conclude that the normal stomach secretes the Cl ion in about the same concentration as found in blood. If this is correct increased gastric acidity would result from increased ionization of the blood chlorides alone.

The bile acids are known to have general cytolytic action. The initial stages or a slight degree of such cytolysis may increase the excitability of cells through changes in cell permeability. If in obstructive jaundice there is enough of bile acids in the blood to have such effects on the acid secreting cells, we might expect an increased acidity in the gastric juice, others factors being constant.

Since the increased gastric acidity fluctuates it is possible that the rhythm parallels the liver changes. The increased acidity of gastric juice soon after the production of obstructive jaundice is possibly associated with increased hepatic and cystic pressure. The fluctuations afterward noted in the acidity of the gastric juice may possibly be related to the intrahepatic and cystic pressure.

The diminished secretion of gastric juice may possibly be explained as due to depression and atonia. Other factors which cannot be evaluated are progressive ascites, edema and nephritis. Thus the quantity of secretion may be limited without limiting the quantity of acid formed.

SUMMARY

1. Intravenous injection of $\frac{1}{8}$ to $\frac{1}{2}$ cc. of bladder bile per kilogram of body weight in dogs causes an immediate but temporary inhibition of gastric motility (hunger), and greatly diminishes the gastric secretion following a test meal. The inhibitory effect upon gastric secretion is more prolonged than upon gastric motility.

2. Chronic obstructive jaundice is followed by a decrease of the motor activity of the empty stomach, a decreased rate of gastric juice secretion, and an increased acidity of the gastric juice.

The writers wish to express their appreciation to Dr. O. H. Horrall who performed the ligation operations.

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EPINEPHRIN AND HYPERGLYCEMIA

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In the course of other work it became possible to test in part the hypothesis recently put forward by Cori and Cori (1928) with respect to the effect of epinephrin on the carbohydrate of the body. As I understand these authors, they think that after the administration of epinephrin less sugar passes from the blood to the muscles, and at the same time more muscle glycogen is oxidized, while part of the lactic acid produced in this oxidation is reconverted in the liver to glycogen. The net result of this process is an increase in liver glycogen and a decrease in muscle glycogen. The chief cause of the hyperglycemia is the decreased "utilization" of the blood sugar. They also state that blood sugar derived from liver glycogen is utilized in the muscles. Their experimental evidence, apart from respiratory experiments, consists in analyses of the liver and the rest of the body three hours after a dose of epinephrin.

In view of the fact that other authors (e.g., Choi, 1927–28) have found no effect on muscle glycogen from epinephrin, it seemed possible that this discrepancy might be due to the length of time elapsing between the administration of epinephrin and the analyses.

I used cats, previously starved for 24 hours, under amytal anesthesia. While this anesthetic (and the operative procedure) undoubtedly affect carbohydrate metabolism, the effect is comparatively slight. The procedure was as follows. After taking samples for blood sugar, amytal was given subcutaneously in two doses of 60 and 15 mgm. per kgm. body weight with an interval of half an hour between them. After about 3 hours the abdominal cavity was opened and a sample of liver removed of about 10 grams. Then a portion of about the same weight of the right semi-membranosus was removed. One and a half hour later another portion of liver and a piece of the left semi-membranosus were taken. It is known that the difference in glycogen content of corresponding muscles is very slight. Epinephrin was given (except to the controls) immediately after the first samples were taken. The glycogen in the various samples was determined by Pflüger's method, and the final sugars as well as the blood sugars by the method of Hagedorn and Jensen.

Results are given in the protocols. They may be summarized thus:

Protocols

TIME	BLOOD SUGAR, MGM. PER 100 CC.	MUSCLE GLYCOGEN AS GRAM GLUCOSE PER 100 GRAMS	LIVER GLYCOGEN AS GRAM GLUCOSE PER 100 GRAMS
	Cat A. 3.47 kgm.		
10:50 a.m.	95		
11:55	123		
12:55 p.m.	150		
1:40	140		
2:00		0.95	2.6
2:55	144		
3:35		0.99	1.6
	Cat B. 3.70 kgm.		
11:10 a.m.	84		
11:55	99		
1:50 p.m.	126		
2:10		1.09	2.9
2:50	142		
3:30		0.97	2.3
	Cat C. 2.65 kgm.		
11:14 a.m.	109		
11:45	99		
12:35 p.m.	115		
1:55	107		
2:10		0.93	2.4
2:15	Epinephrin—1 cc. per kilogram subcutaneous		
2:30	227		
2:45	263		
3:00	247		
3:15	233	0.90	0.08
	Cat D. 2.93 kgm.		
10:55 a.m.	92		
11:45	106		
2:15 p.m.	77		
2:30		1.3	1.1
2:35	Epinephrin—1 cc. per kilogram subcutaneous		
2:50	270		
3:50	200	1.0	0.08

The muscle glycogen in no case shows a significant change. In the animals not receiving epinephrin the liver glycogen falls respectively to $\frac{3}{5}$ and $\frac{2}{3}$ of its original values. In animals receiving epinephrin there is less than $\frac{1}{16}$ of 1 per cent of glycogen in the liver; i.e., it fell to ^{1} and $\frac{1}{25}$. Moreover in all cases there is some hyperglycemia, and, roughly speaking, the greater the hyperglycemia the greater the loss of liver glycogen.

Discussion. My results thus differ from those of Cori and Cori in two respects, viz.: 1, I find a great loss of liver glycogen where they find an increase, and 2, I find no change in muscle glycogen where they find a decrease. How may these be reconciled? Four possibilities present themselves: difference in species, differences due to the anesthetic, difference in dosage, and in time relationships. Of these the first two seem improbable. Cori and Cori emphasize the fact that their dosages were small enough to be non-glycosuric. However, it does not seem probable that increasing the size of the dose will produce the opposite change. It is difficult to compare the time relations except to note that in my case the epinephrin had acted for $1\frac{1}{2}$ hour and in the Coris' experiments it had acted for 3 hours. An inspection of the protocols will show that my analyses were made a short time after the blood sugar had reached its maximum value. (Data on this point are not given by the Coris.) One would expect to get the greatest effect from epinephrin about the time of maximum blood sugars, for after this time its action has diminished and the changes occurring are secondary to the disturbances in carbohydrate distribution produced by epinephrin. I believe that the Coris' figures indicate chiefly these secondary changes, and suggest that their theory be modified in the following way. The excess of sugar in the blood after epinephrin comes from the liver. The extent of the hyperglycemia is probably increased due to a decrease in the amount of sugar passing from the blood to the muscles, as suggested by Achard, Ribot and Binet (1921). Calculations based on my experiments tend to bear this out. Cat B lost 0.6 per cent of the glycogen in the portion of the liver remaining (72 gm.) i.e., 430 mgm. glucose. Reckoning the blood as 8 per cent of the body weight, the greatest amount of excess glucose at any time in the blood was 142 − 126 = 16 mgm. per cent or 40 mgm. altogether. Hence nearly 400 mgm. of glucose passed out of the blood in the time. Similar calculations on cat D show that here only 300 mgm. glucose left the blood in spite of much greater hyperglycemia. Is this due to decreased blood flow through the tissues? Probably not, for the increase in blood pressure is very slight after subcutaneous doses. It may be ascribed provisionally to altered permeability. Thus the primary effect of adrenalin is to increase the passage of glucose derived from liver glycogen into the blood and to decrease the passage of glucose out of the blood. The later effects probably result in a diminution of muscle glycogen and an increase in that of the liver.

The increase in metabolism probably occurs early, although satisfactory data on the cat are difficult to obtain. If this is so, the increased metabolism (which in any case involves much more than carbohydrate oxidation) does not appear to be at the expense of the muscle glycogen. Further work, however, is necessary to enable one to follow all the carbohydrate changes.

SUMMARY

One and a half hours after the administration of epinephrin to cats under amytal anesthesia the glycogen content of the muscles is unaltered while that of the liver has been reduced almost to zero.

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THE EFFECT OF LOWER ABDOMINAL SYMPATHECTOMY ON THE OESTROUS CYCLE

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The recent interest in the effects on function of removal of parts or all of the sympathetic system, that has been prominent in this laboratory, led us to undertake an investigation into the possible relation of that system to the recurrence of oestrus. As subjects we used adult white rats, from six to seven and a half months old. They were studied for a period of three and a half months. During that time they were fed on mixed grain and dog biscuit, with meat scraps once a week and lettuce twice a week. Every day the stage of the oestrous cycle was watched by means of a vaginal smear, according to the method described by Long and Evans (1922).

After a preliminary stage of at least six weeks, the animals were anesthetized with amytal,[1] and under a dissecting microscope and with proper aseptic care, the abdominal sympathetic trunks were removed from the level of the renal veins to well down in the pelvis. In control operations, which lasted about as long as the complete operations, the abdominal trunks were as fully exposed as in the experimental cases but they were not excised.

We excised the sympathetic trunks in 26 animals; 5 did not recover from anesthesia, 1 fell ill soon after the operation and died; 1 did not show an oestrous cycle either before or after the sympathectomy; 1, which was fairly regular before the operation, did not show any resting stage thereafter; and when the remaining 18 animals were autopsied at the end of the tests, 2 were found in which it was thought that the right sympathetic trunk could be identified. Sixteen animals were left as satisfactory for the purposes of the experiment. Of the 8 control rats 2 failed to recover from the effects of the amytal and in 1 only a single post-operative test could be made; 5 remained.

The results of the observations on both the control series and the sympathectomized series are shown in the accompanying table. The average interval between recurrences of oestrus was prolonged in both series, but

[1] One gram of amytal was mixed in a beaker with 8.85 cc. of tenth normal NaOH; the beaker was placed in a hot water bath for about 30 minutes during which the mixture was repeatedly stirred; care was exercised that the temperature did not rise above 80°C. It was then cooled, diluted to 20 cc. with water, and filtered. A dose of 1.5 cc. per kilo subcutaneously was used to produce anesthesia.

TABLE 1

Showing intervals between oestrous cycles in control animals operated upon but not sympathectomized and in experimental animals from which the lower abdominal sympathetic strands were removed

The time of operation is indicated in each instance by the parentheses; the figure to the left in parenthesis designates the interval, in days, since the last cycle before operation, and that to the right the interval after the operation before the next cycle

NUMBER	AGE	INTERVALS (DAYS) BETWEEN CYCLES Pre-operative		Post-operative	PRE-OPERATIVE AVERAGE	POST-OPERATIVE AVERAGE	REMARKS
		Control animals					
	months						
36	6	11, 3, 5, 4, 4, 3, 4, 4, 4	(2–3)	5, 4, 4, 6	4 40	4.40	
37	6	4, 5, 4, 5, 3, 5, 3, 4, 4, 4, 4	(3–1)	5, 6, 5, 6	4.00	4.60	
38	6	5, 7, 3, 3, 3, 3, 5, 6, 5, 4	(2–12)	4, 4	4.18	6.66	
40	6	4, 5, 4, 4, 4, 4, 4, 4, 4, 4, 4	(1–4)	4, 5, 4, 7+	3.83	4.80	Vaginal discharge 2 days post-operative
44	6	4, 5, 5, 4, 4, 3, 4, 4, 5, 3,	(5–0)	5, 4, 4, 7	4.18	5.00	
Average of control animals					4.12	5.09	
Average prolongation of interval					0.97 day		
		Experimental animals					
42	6	13, 5, 4, 4, 6, 4, 4, 3	(1–6)	4, 5, 9	5.50	6.00	Vaginal discharge 2 days post-operative
43	6	7, 6, 5, 4, 7, 4, 3, 7, 4, 3	(9–6)	12, 4	5.36	7.33	
45	6	5, 4, 4, 5, 4, 4, 4, 4, 4, 4	(2–3)	5, 5, 5, 8+	4.00	5.20	Vaginal discharge 1 day post-operative
46	6	5, 4, 4, 4, 4, 4, 4, 4, 5, 4	(2–11)	4, 4, 4	4.00	5.20	
47	6	5, 5, 3, 4, 5, 4, 4, 4, 4, 4	(2–2)	6, 4, 3, 4, 5	4.00	4.00	Vaginal discharge 2 days post-operative

TABLE 1—*Concluded*

NUMBER	AGE	INTERVALS (DAYS) BETWEEN CYCLES Pre-operative		Post-operative	PRE-OPERATIVE AVERAGE	POST-OPERATIVE AVERAGE	REMARKS
			Experimental Animals				
48	6	3, 4, 4, 4, 4, 4, 4, 4, 4	(1–4)	4, 4, 3, 15	4.00	6.00	
53	6	4, 5, 2, 5, 5, 3, 4	(3–2)	5, 5, 4, 4, 4, 4, 4, 4	3.87	4.00	
54	6½	4, 4, 4, 4, 3, 4, 5, 3, 3, 5, 4	(4–1)	4, 4, 4, 4, 4, 4, 4, 5, 5	3.91	3.90	
55	6½	4, 5, 5, 3, 5, 4, 5, 5, 4	(0–6)	5, 5, 4, 5, 5, 6, 5, 6	4.44	5.22	
58	6½	5, 4, 5, 5, 5, 5, 5, 5, 4	(1–4)	5, 5, 5, 5, 5, 6, 5, 5	4.40	5.00	Right cystic kidney at autopsy
59	?	4, 4, 4, 4, 4, 5, 3, 3, 4	(3–7)	9, 5, 3, 5, 5, 3, 4, 4, 6	3.80	5.10	Not "smeared" for 2 days in 9-day period. Vaginal discharge 3-5th day post-operative
62	?	4, 5, 5, 5, 5, 4, 5, 4	(3–1)	4, 5, 3, 5, 4, 4, 3, 4, 5, 5, 6	4.44	4.08	
63	7½	5, 5, 4, 5, 3, 5, 4, 3, 5, 4, 5	(0–4)	4, 4, 4, 4, 5, 5, 3, 5	4.36	4.22	
65	7½	4, 4, 6, 4, 5, 4, 6, 3, 5, 3	(3–3)	5, 5, 4, 3, 6, 5, 10	4.27	5.12	
68	6	17, 5, 7, 4	(6–5)	3, 4, 6, 12, 5, 4, 8	7.60	5.87	
69	6	3, 4, 7, 4, 6, 4, 3, 6	(3–3)	6, 5, 4, 5, 4, 5, 7, 5, 5	4.44	4.90	Vaginal discharge 29–30th day post-operative
Average of experimental animals					4.52	5.07	
Average prolongation of interval					0.55 day		

it was slightly more prolonged in the control series (0.97 day) than in the series in which the complete operation was performed (0.55 day). If rats 38 and 46 are excluded because of the unusually long delay in the return of oestrus, after the operation, the average delay in the two series is almost the same.

As noted in the table, some rats in both series had a transient purulent discharge from the vagina, usually a short time after the operation. We did not find that this condition materially interfered with the identification of the stage in the oestrous cycle.

In nearly every rat from which the sympathetic trunks were removed, the operation was followed by an apparent primary loss of tone of the vaginal walls, for the aperture gaped open. In the course of 4 to 6 days there was a gradual return to the normal pre-operative state.

From our observations we conclude that removal of the lower abdominal sympathetic trunks in the white rat has no noteworthy effect on the recurrence of the oestrous cycle other than that resulting from a similar operation without sympathectomy.

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STUDIES IN THE NUTRITION OF THE WHITE MOUSE

V. The Experimental Production of Rickets in Mice

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In a preceding paper of this series (1926a) one of us reported a study of feeding unbalanced salt mixtures to mice in an attempt to produce rickets in these animals as has been done in rats under similar conditions. One group of mice was fed the Sherman-Pappenheimer diets 84 and 85 (1921) for 120 days, at which time gross and histological examinations of the bones did not reveal any rachitic changes, due no doubt to spontaneous cures having taken place during the period of study. Other groups were given the low calcium-high phosphorus and low phosphorus-high calcium diets of Osborne, Mendel and Park (1923). At the end of 28 days on these diets no evidence of rickets was observed.

We were not quite satisfied at the time that these results were correct, as we could see no reason why rats and mice should react differently under similar conditions of diet and treatment. However, we could draw no other conclusion but that the mice did not develop rickets, or if they did that spontaneous cures must have taken place before the histological studies were begun after four weeks on the experimental diets.

In our present study of this question we found that the latter view is the correct one. Mice develop rickets and cures take place much quicker than is the case in rats, and our previous conclusion that the mouse does not develop rickets, in the light of the results to be presented below, is no longer tenable.

Experimental. Young mice, 3 weeks of age and raised in this laboratory were used. The technique of feeding and caging has been described elsewhere (1926b). In most cases x-ray examinations were taken of the animals the first day of the experiment and at stated intervals thereafter, and at the end of the experimental periods, usually 7, 13 and 27 days, sections of a radius and rib were made for histological study. No record of food intake was possible as mice tend to scatter the diets, but as the weights of all animals showed slight gains we must conclude that an adequate food intake was maintained.

TABLE 1

MOUSE NUMBER	DIET	DURATION OF FEEDING TEST	WEIGHT	X-RAY EXAMINATION	HISTOLOGICAL EXAMINATION
		days	*grams*		
52	Steenbock	1	8.5	Normal	
		8	9.0	?	Rib-moderate rickets
53	Steenbock	1	9.0	Normal	
		8	9.0	Rickets +	Rib-slight rickets
18	Steenbock	1	6.5	Normal	
		7	7.5	Rickets +	
		14	9.0	Rickets ++− healing	Rib and radius—rickets
19	Steenbock	1	7.0	Normal	
		7	9.5	Rickets +	
		14	10.0	Rickets ++− healing	Rib and radius—rickets, some healing
64	Steenbock	1	9.0	Normal	
		7	9.0	Rickets +	
		10	10.5	Rickets +	Rib and radius—slight rickets
		13	10.5	healing	Osteoporosis ++
66	Steenbock	1	7.0	Normal	
		7	8.0	Rickets +	
		10	8.5	Rickets +	Rib and radius—slight rickets
		13	8.7		
72	Steenbock	1	9.0	Normal	
		7	11.0		
		13	13.0		
		20	15.0	Normal	Rib and radius—nearly normal
		27	17.0		Slight osteoporosis
74	Steenbock	1	10.0	Normal	
		7	13.0		
		13	13.0		
		20	13.0		
		27	13.0	Normal	Rib and radius—osteoporosis

RESULTS. *Group 1.* The mice of this group received the diet of Osborne, Mendel and Park with a low phosphorus-high calcium salt mixture. Very little growth was obtained and at the end of 7 days there was evidence of rickets, which had healed at 28 days. All the surviving animals of this group developed xerophthalmia between the fiftieth and sixtieth days. This phase of the subject will be presented in a later publication.

Group 2. We next desired to use diet 2965 of Steenbock and Black (1925) to produce rickets. It consisted of the following:

	grams
Yellow corn meal	76
Wheat gluten	20
Calcium carbonate	3
Sodium chloride	1

The results obtained with the mice of group 1 above demonstrated that one must begin to look for evidence of rickets after 7 days on the experimental diet. This fact was not appreciated in our earlier work, due to the fact that we wished to begin histological studies at 28 days, the usual time at which rickets is pronounced in rats. The results in table 1 show that rickets begins at 7 days and is the most severe at 14 days, at which time, however, some early indications of healing are in evidence.

Group 3. We next studied a large number of animals on the Steenbock-Black diet, making examinations, as above, at weekly intervals on different lots of mice. Since the results were practically the same as shown in table 1, it will suffice to describe the results obtained very briefly. At the end of 6 to 8 days there was evidence of slight rickets, which was much more pronounced at 14 days. Again at this time there was evidence of healing in several animals. At 27 days all animals were practically normal, with the exception that osteoporosis was present in many cases at this time. The histological examination at the end of each of the weekly periods confirms in practically all cases the x-ray findings.

The healing observed was not due to starvation since all of the animals had abundant food before them at all times. Growth was very poor in all cases.

CONCLUSIONS

Mice fed the Osborne, Mendel and Park diet, containing a low phosphorus-high calcium salt mixture, develop rickets as early as 7 days, with evidence of healing about 28 days. Those fed on the Steenbock-Black diet 2965 also developed rickets at 7 and 14 days, with healing at 20 and 27 days. Osteoporosis was present in many cases, especially after healing had taken place.

We wish to take this opportunity to thank Dr. Harry Goldblatt of the Department of Pathology for the diagnosis of the x-ray plates and histological sections, and Miss Elspeth MacCallum of the Pathological Laboratory of City Hospital, for making the sections of bones for histological study. We also thank Dr. H. J. Gerstenberger and Mr. Shields, of the Babies' and Children's Hospital, for the x-ray examinations.

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EFFECT OF ANOXEMIA, CARBON DIOXIDE AND LACTIC ACID ON ELECTRICAL PHENOMENA OF MYELINATED FIBERS OF THE PERIPHERAL NERVOUS SYSTEM

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The present paper deals with changes in the physiological characteristics of certain myelinated nerve fibers[2] when deprived of oxygen or treated with an excess of carbon dioxide or lactic acid. Recently with the cathode ray oscillograph as a recording apparatus Erlanger, Gasser and Bishop in this laboratory have recorded the form of the action potential at the stimulus and that of the compound conducted action potential. They have proven the presence of fiber groups which differ in thresholds and conduction rates. It therefore seemed advisable to make a unified and more or less coördinated study of the effect of anoxemia, carbon dioxide and lactic acid on the action potential using the cathode ray oscillograph as a recording mechanism. In part there is a repetition of work by other investigators but chief emphasis has been placed on the modification of those details of nerve activity which are revealed by the newer and more precise technique of this laboratory.

METHOD. In view of the work of Kato (1924) the usual experimental method of subjecting only a portion of the nerve under observation to the influence of a modifying agent was avoided. Instead, the whole preparation was treated uniformly by using a nerve chamber sufficiently large to accommodate the entire nerve, and all stimulating and recording electrodes. Nerve preparations as long as 10 to 12 cm. could be employed. The materials utilized in the experiments were the sciatics of green frogs and bullfrogs and bullfrog nerve roots. Care was taken to apply all electrodes at uniform stretches of nerve without branches, though in long nerves conduction past cut branches could not be avoided. To render a nerve

[1] Much of the work reported in this paper was accomplished while the author was a fellow of the National Research Council in Medicine.

[2] The fibers studied in these experiments are those characteristic of the peripheral nervous system and giving rise to the *alpha*, *beta*, *gamma* and *delta* potential groups of Erlanger and Gasser which have certain properties in common distinguishing them from other types of fibers existing in these nerves. Fibers in these nerves characteristic of the involuntary nervous system will be reported upon in a subsequent paper.

anoxemic commercial hydrogen or nitrogen over 99.7 per cent pure, was blown through the nerve chamber in a constant stream. Gas analyses showed that in 3 to 4 minutes the chamber contained no air. Equally pure commercial carbon dioxide was applied in a similar manner. All gases were bubbled through water to control humidity. Lactic acid was employed in only one concentration, 250 mgm. per 100 cc. of frog Ringer's solution. It was applied to the nerve by means of a dropper which permitted uniform application. All experiments were carried out at room

TABLE I A

Experiment (a) to show change in threshold and amplitude of action potential during depression by nitrogen and recovery in presence of oxygen; green frog sciatic June 1, 1928

TIME	CURRENT FOR THRESHOLD RESPONSE	CURRENT FOR MAXIMAL RESPONSE	AMPLITUDE MAXIMAL ACTION POTENTIAL SHUNTED 110,200 OHMS	REMARKS
			mm.	
8:50	76	472	46	Normal
8:51				Nitrogen started
9:00	72	460	50	
9:30	70	480	51	
10:00	96	520	27	
10:30	124	520	15	
10:31				Oxygen started
10:45	100	460	49	
11:15	108	436	51	
12:15	108	432	51	
1:23	102	480	40	
2:45	106	470	30	

Note. Experiment shows a heightened irritability in the early stages of anoxemia. Return to normal threshold does not occur though amplitude returns to normal. In the later stages irreversible degeneration manifests itself. Current in arbitrary units of primary current.

temperature (20 to 26°C.) constant within one degree for any single experiment.

To stimulate, induction shocks 0.06 sigma in effective duration or galvanic stimuli 0.1 sigma in duration were employed. The induction shocks were applied through platinum electrodes, the galvanic stimuli through nonpolarizable calomel electrodes. As leads to the recording apparatus platinum electrodes wound with silk thread served in most experiments after it was demonstrated that the results were identical to those obtained under like circumstances with nonpolarizable electrodes. The absence of detectable polarization of such platinum electrodes results from the use of an amplifier with a high input resistance.

Threshold of nerve, as affected by anoxemia, carbon dioxide and lactic

acid. Threshold measured by the current required for a minimal response is the nearest to a quantitative criterion we know of the irritability or reactivity of nerve. Threshold depends on the physical reactance of nerve as well as on its metabolic state and it is therefore not necessarily always a true index of the latter. In air, the current required for such a response remains fairly constant, the threshold decreasing approximately 5 to 10 per cent in 3 to 4 hours. The degree of normal change is thus not sufficient to impair the value of results dependent upon other agencies. The time required to raise the threshold of a nerve or nerve root to the point of extinction of its action potentials by anoxemia varies considerably with

TABLE 1 B

Experiment (b)—similar to experiment (a) with carbon dioxide as depressing agent; green frog sciatic May 31, 1928

TIME	CURRENT FOR THRESHOLD RESPONSE	CURRENT FOR MAXIMAL RESPONSE	AMPLITUDE MAXIMAL ACTION POTENTIAL SHUNTED 110,200 OHMS	REMARKS
			mm.	
5:41	84	304	51	
5:42				CO_2 started
5:45	112	384	48	
5:47	188	412	28	
5:49	212	416	23	
5:50	224	417	20	
5:51				Oxygen started
5:56	192	360	35	
6:60	144	330	40	Experiment shows
6:10	106	328	45	threshold return
6:15	92	316	45	above normal, ampli-
6:20	90	308	45	tude just below
6:25	84	304	43	normal
6:30	83	288	42	
9:30	78	284	50	

the preparation (2 to 7 hours). A usual time is $2\frac{1}{2}$ to 3 hours (table 1A). It is quite often found that in the first 15 to 30 minutes of the experiment the threshold of a nerve preparation is definitely lowered, a condition usually associated with an increased amplitude of action potential, an increased conduction rate and invariably with an increase in the absolutely refractory period. Such a lowering of threshold is considered analogous to that developing in excised nerves in air or on standing in Ringer's solution. In general it can be said that nerve roots are more quickly depressed by the conditions imposed than are nerve trunks. Pure carbon dioxide causes a very rapid raising of threshold in both nerve trunks and nerve roots (table 1B). By it the threshold may be more than doubled in half

an hour. After this first rapid increase the rise in threshold slows considerably to a rate more comparable to that resultant from hydrogen or nitrogen. Lactic acid, more effective in the concentration used, rapidly raises the threshold of a nerve or nerve root to extinction. That the depressing action of carbon dioxide is in part different from that of nitrogen can be quite strikingly demonstrated by the following experiment. A nerve in the early stages of depression by carbon dioxide can be quickly restored almost to normal by surrounding it with nitrogen. To illustrate, a nerve with a normal threshold of 80, action potential amplitude of alpha wave 46 mm., a conduction rate of 61.94 m. per second and absolutely refractory phase of 0.83 sigma was in 27 minutes depressed by pure CO_2 so that its threshold was 208, action potential amplitude 28 mm., conduction rate 35.35 m.p.s. absolutely refractory period 1.45 sigma (fig. 1, 10). It was then surrounded with nitrogen containing no detectable oxygen by analysis with the Henderson modification of the Haldane apparatus. In 10 minutes the threshold was 160, amplitude of action potential 41 mm. conduction rate 38.25 m.p.s., absolutely refractory period 0.98 sigma. In 10 minutes more further recovery occurred, the threshold being 96, amplitude of action potential 47, conduction rate 45.16 and absolutely refractory period 0.83 sigma (fig. 1, 10′). In 35 minutes the depressing action of anoxemia manifested itself, the threshold being 108 with corresponding changes in the other measurements.

Effect of continuous stimulation on the rate of depression of threshold in anoxemic nerves. Fenn (1927) and Gerard (1927) found an increased oxygen consumption in nerve as a result of activity. Gerard, Hill and Zotterman (1927) determined the increased heat production on stimulation. Consequently it was of interest to know the effect of continuous stimulation on the threshold of nerves being depressed by anoxemia. To accomplish this the two sciatic nerves of the same frog were mounted together in the nerve chamber.[3] One of the nerves was stimulated continuously with approximately maximal induction shocks 10 to 12 times a second and the other was stimulated briefly only at the time a record was necessary. In oxygen a normal nerve is little influenced by stimulation at such a rate. After establishing the technique the results were uniform in showing a raising of threshold as a result of stimulation (table 2). A type experiment is presented in graph I. The current required to elicit an action potential of constant amplitude increases more rapidly in the stimulated nerve than in the nonstimulated and the irritability of the stimulated nerve is completely depressed in a shorter time. The difference is consistent with

[3] Especial care was taken in the dissection of such preparations and the branches were not cut closely to their point of exit from the nerve. Experiments demonstrated that depression in irritability occurs more rapidly in anoxemia at those points where branches cut close to the nerve trunk are found.

the results of Gerard (1927) who found what he termed the "oxygen debt" (in nitrogen) greater in activity than during rest. During the experiments it was frequently noted that if stimulation of the constantly stimulated nerve was interrupted for 2 to 3 minutes its threshold fell perceptibly. To cite a typical example—a nerve in hydrogen continuously stimulated for 70 minutes 10 times a second had a threshold stimulus value of 156. After 3 minutes' *interruption* of stimulation the threshold stimulus value fell to 112 (recovery from fatigue). On the other hand, the threshold of the nonstimulated nerve during *3 minutes of stimulation* fell from 96 to 84. The latter is interpreted as an illustration of "treppe" on stimulation of a rested nerve depressed by anoxemia. The ability of nerves to recover from partial anoxemia on restoration of oxygen and washing with Ringer's is not impaired by such prolonged stimulation. There was never any evidence of a change in the form of the action potential as a result of fatigue. It is believed that changes found by Tigerstedt (1912), Boruttau (1901) and other investigators were the result of differential changes in conductivity of the fiber groups.

Differential changes in the alpha, beta and gamma and delta fiber groups under anoxemia and carbon dioxide. It is found that the delta and gamma fibers first fail to respond to maximal or supramaximal stimuli; then the beta fibers and, finally, the alpha fibers (table 3). The order of depression

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Fig. 1. Films 1, 2, 3 and 4 action potentials green frog sciatic. No. 1 threshold 78, maximal cathodal response with low anodal response. No. 2 threshold 74, action potential after 10 minutes in an atmosphere of nitrogen; note increase in amplitude with lower threshold and nearly complete loss of anodal response. No. 3 threshold 128, maximal action potential after 100 minutes in nitrogen; crest earlier and narrower, duration of cathodal response shortened because of loss of anodal effect. No. 4 threshold 108, maximal action potential after 90 minutes in oxygen; amplitude the same as the film 2, duration same in film 3.

Films 5, 6, 7 and 8 action potentials bullfrog sciatic. No. 5 threshold 80, normal submaximal action potential. No. 6 threshold 74—submaximal action potential after 15 minutes in an atmosphere of hydrogen. Note shorter duration in spite of lower threshold; crest is earlier and narrower, amplitude is greater. No. 7 normal conducted action potential. No. 8 conducted action potential corresponding to film 6 taken near the stimulus. Note increase in conduction rate with lower threshold in early stage of anoxemia.

Films 9, 11 and 12, submaximal action potentials bullfrog sciatic. No. 9 action potential of normal nerve. No. 11 and 12 action potential after treatment with lactic acid (250 mgm. per 100 cc. frog Ringer's solution). Note decrease in amplitude and shortening of duration of equal degree in films 11 and 12 as compared to film 9.

Films 10 and 10′. Conducted action potentials bullfrog sciatic. No. 10 after 27 mm. in pure carbon dioxide, 10′ the same after 20 mm. in atmosphere of nitrogen over 99.7% pure. Note return to normal amplitude and increase in conduction rate in nitrogen.

Films, 2, 4, 5, 6, 8, 9, 11 and 12 have been retouched somewhat to aid reproduction.

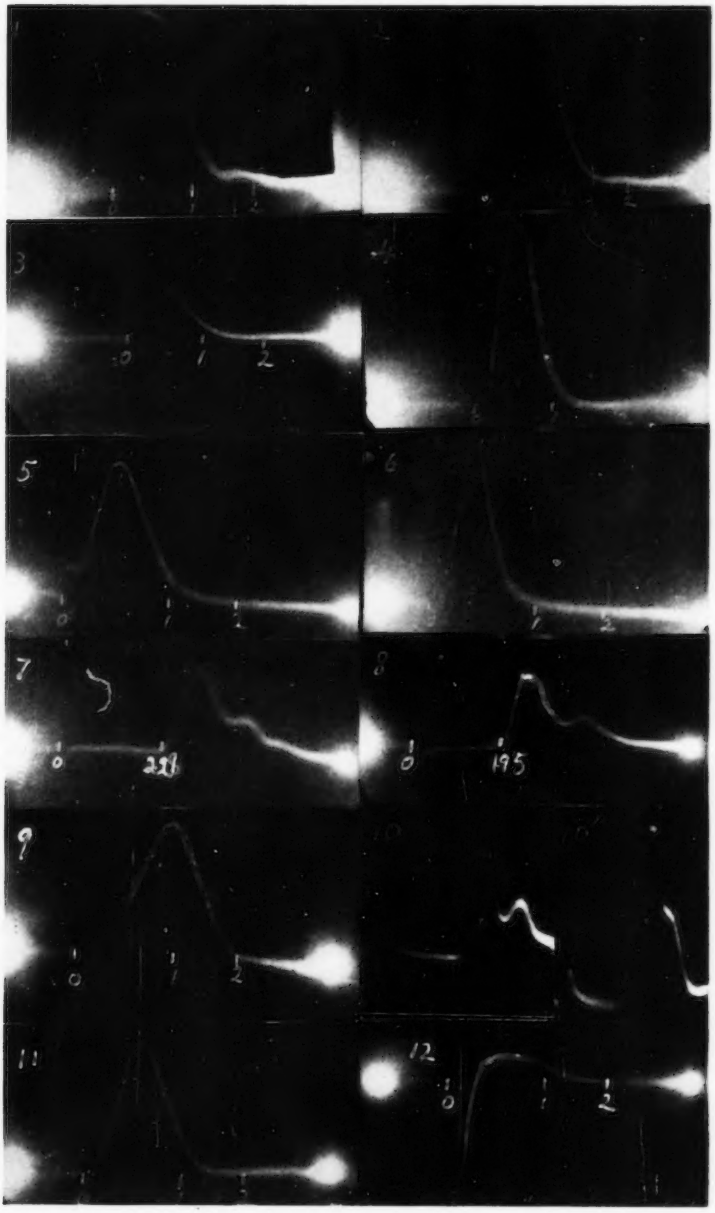


Fig. 1

is thus inversely that of the fiber diameters. This is the result expected to follow from a consideration of the purely physical factors involved. The ratio of surface area to volume of the smallest, or delta, fibers is greater than it is for the largest or alpha fibers. The per cent of the volume within a given distance from the surface is greatest in the smallest fibers. Consequently the oxygen from within the smallest fibers would be expected to diffuse out more rapidly than from within the largest fibers, also there might be expected to be less reserve material in relation to active surface. Threshold being in large measure dependent upon the oxygen supply, could then be depressed most rapidly in the smallest fibers.

It will be noted in the results (table 3) that the current required for a threshold response in all fiber groups is consistently increased with increasing degrees of anoxemia. The current required for a *maximal* response of the fibers capable of responding at all at a given stage of depression may decrease. This is quite consistent with the observation that the fibers with the highest threshold are first depressed to extinction. When these fibers fail to respond the current required to elicit a maximal response from those remaining may be actually less than for the previous maximum.

Recovery of threshold after anoxemia, carbon dioxide and lactic acid. In approximately 50 per cent of nerves recovery to normal is possible. No definite reason for the only partial recovery of the others can be given. Other observers have also found it difficult to secure complete recovery (Gerard, Hill and Zotterman, loc. cit.). In those experiments where depression is not carried to completion and in the briefer experiments with carbon dioxide the chances for complete recovery are always better.

Fig. 2. Films 1, 2, 3, 4, 5, 6, 7 and 8, action potentials bullfrog nerve root 5 mm. conduction. No. 1 normal nerve. Note cathodal and anodal responses. No. 2 maximal unshunted normal cathodal response with elimination of anodal response. No. 3 normal nerve, same as no. 1 but stimulus weaker. No. 4 same as no. 2 shunted to amplitude of no. 1. No. 5 normal single cathodal response, stimulus below threshold for anodal response. No. 6 maximal response after 15 minutes in atmosphere of carbon dioxide. Note absence of anodal response. Duration less than that of no. 5 because spread no longer occurs but of the same duration as no. 4. No. 7 maximal shunted response after 50 minutes in oxygen. No. 8 maximal unshunted response after same time in oxygen as no. 7. Note duration of no. 7 similar to that of no. 4; duration of no. 8 similar to that of no. 2.

Films 9, 11 and 13. Maximal action potentials bullfrog sciatic 2.5 mm. conduction. No. 9 normal nerve threshold 78. No.11 threshold 104 after 11 minutes in carbon dioxide. No. 13 threshold 72—, after 25 minutes in oxygen. Note amplitude and area of no. 13 greater than those of no. 9, the unaltered nerve.

Films 10, 12 and 14. Maximal conducted action potentials corresponding to films 9, 11 and 13. Note in spite of lower threshold and increased amplitude of no. 13 the conduction rate illustrated in film 14 is still below the normal film 10. Films 2, 8 and 9 have been retouched for reproduction.

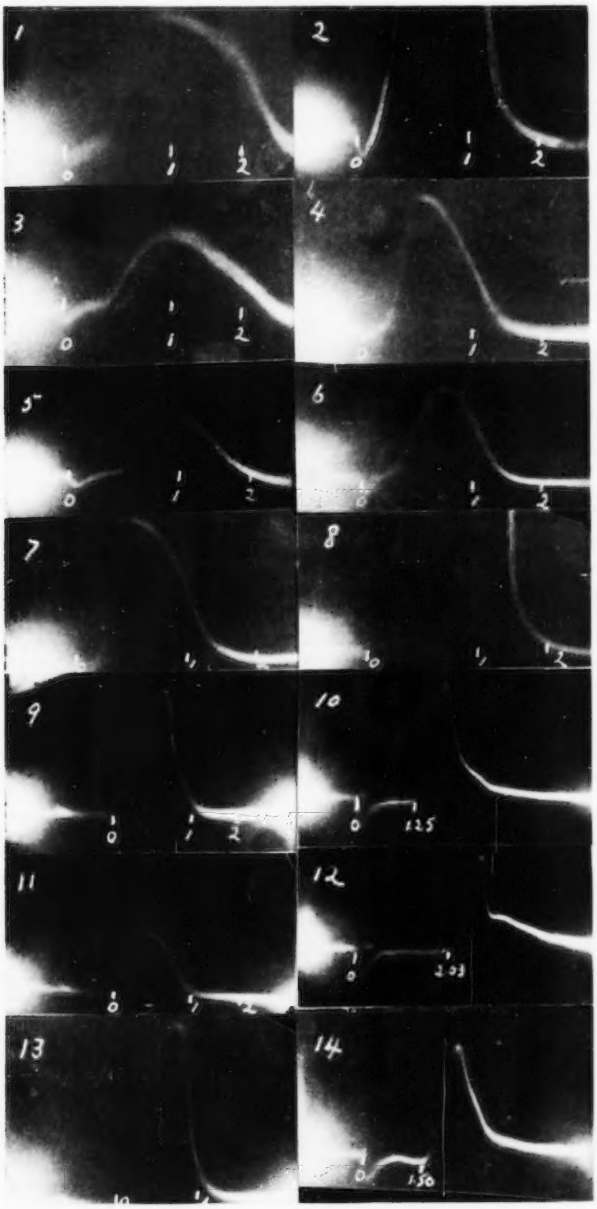


Fig. 2

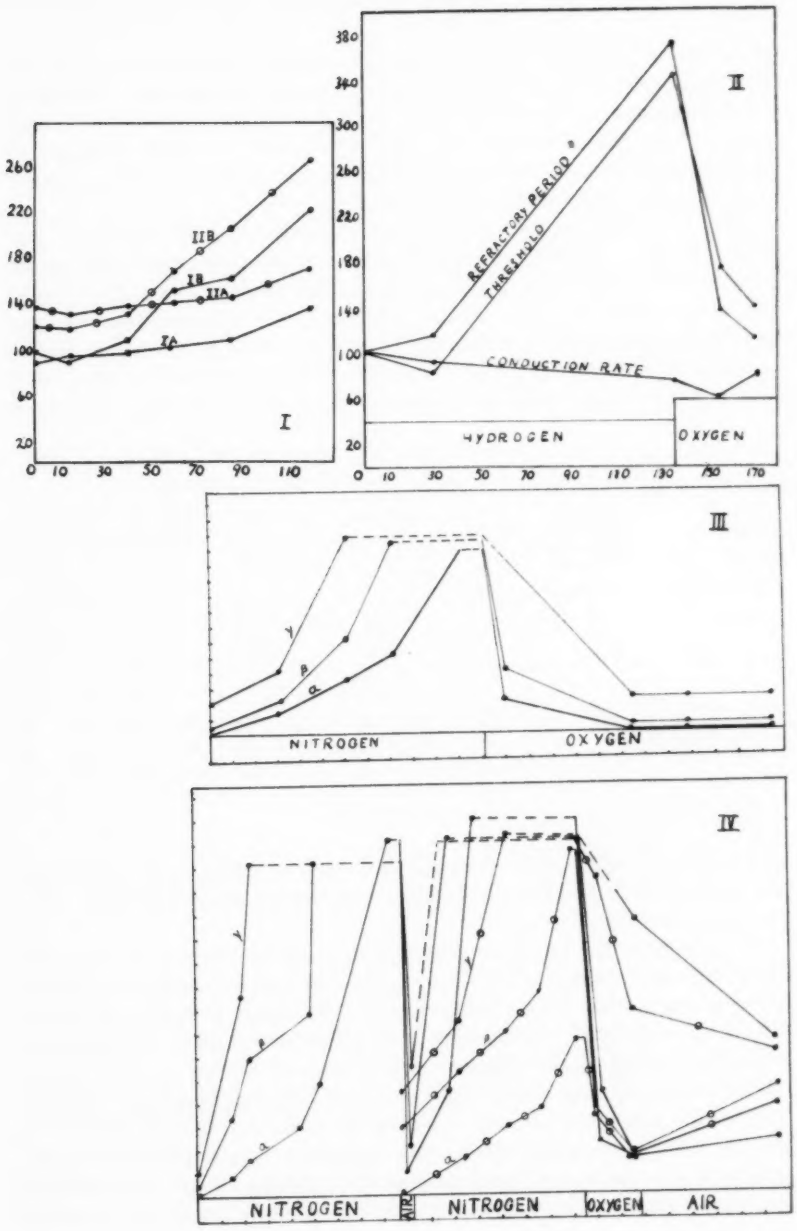
Not infrequently the current required for the minimal or maximal response may finally be less in the revived nerve than it was for the normal nerve (table 1 B). In spite of the rather wide variation possible in different nerves it is certain that depression of threshold can be delayed by frequent washing of a nerve with Ringer's solution even though this is oxygen-free, and a nerve depressed to extinction may temporarily show partial recovery after such treatment, findings similar to those of Thörner (1910). The rate of recovery of irritability in nerves is undoubtedly hastened by washing with Ringer's solution. The rate of recovery of irritability upon readmission of oxygen is much more rapid than the rate of its depression.

Graph I. Changes in threshold of non-stimulated and constantly stimulated nerve in anoxemia. IA,—threshold curve nonstimulated nerve. 2A,—threshold curve stimulated nerve. Green frog. Changes in current required for action potential of equal amplitude in nonstimulated and constantly stimulated nerve in anoxemia. IB, curve of nonstimulated nerve, 2B of constantly stimulated nerve. Current in arbitrary units of primary current. Abscissae—Time in minutes. Ordinates—Percentage change in current strength.

Graph II. Changes in threshold, conduction rate and absolutely refractory period of alpha fibers of bullfrog in anoxemia and recovery in oxygen. Shows unusual decrease in conduction rate at onset with a lowering of the threshold and unusual decrease in conduction rate at onset of recovery when threshold and refractory period have already decreased; action potential at this time also definitely increased in amplitude (fig. 2, no. 4); more usually conduction rate increases at both of these times. Abscissa—Time in minutes. Ordinate—Percentage change. Threshold in arbitrary units of primary current.

Graph III. Absolutely refractory period changes of alpha, beta and gamma fibers during anoxemia and recovery in oxygen. Note the differential depression and the differential recovery; nerve not washed with Ringer's solution. Abscissa—Each unit = 10 minutes. Ordinate—Each unit = 0.1 sigma. Dotted lines indicate the level at which the wave became practically extinct. Nerve same as described in table 4, bullfrog III.

Graph IV. Absolutely refractory period changes of alpha, beta and gamma fibers in two bullfrog nerves during anoxemia. The first nerve indicated by plain black lines was depressed to extinction. Then the chamber was opened long enough to mount the second nerve indicated by the circled lines. Nitrogen was again supplied until the beta fibers of the second nerve were depressed practically to extinction. Recovery in oxygen and air was then instituted. Note the differential depression of the fiber groups. Note the rapid but differential recovery of the first nerve fiber groups on opening the chamber to mount the second nerve. Depression in this nerve now much more rapid as compared to time of first depression (no recovery of oxygen reserve). Note the greater difference in "normal" thresholds of the second nerve fiber groups after preservation in Ringer's (both nerves excised at same time). Note slower recovery of the beta and gamma fibers of the first nerve as compared to second. The alpha fiber groups are not entirely comparable as 5 minutes elapsed between the readings. This would really account for the alpha wave of the first nerve having a shorter refractory period than the alpha wave of the second nerve; nerves not washed with Ringer's solution. Abscissa—Time in units of 0.05 sigma. Ordinate—Time in units of 10 minutes.


I
IIB
IB
IIA
IA
II
REFRACTORY PERIOD
THRESHOLD
CONDUCTION RATE
HYDROGEN
OXYGEN
III
NITROGEN
OXYGEN
IV
NITROGEN
AIR
NITROGEN
OXYGEN
AIR

Recovery in the different fiber groups is earliest and most rapid in the alpha group, then the beta group and finally the delta group. The delay in the recovery of the late groups may be accounted for by the longer period of depression beyond the point of excitability which obtained in these experiments. Similarly the beta group is so depressed for a longer time than the alpha group (graph IV).

Absolutely refractory period of nerve as influenced by anoxemia and carbon dioxide. A review of the literature indicates that most investigators have found the refractory period prolonged as a result of anoxemia. Fröhlich (1904) obtained such results. Cooper (1926) thought the recovery time in anoxemic nerve remained unaltered, showing no change in the total refractory period. Her conclusions were based on evidence it is impossible to accept since Kato (loc. cit.) and Davis, Forbes, Brunswick

TABLE 2

Depressing effect of constant stimulation during anoxemia

THRESHOLD STIMULATED NERVE AT START	MINUTES IN HYDROGEN	THRESHOLD STIMULATED NERVE AFTER HYDROGEN		THRESHOLD NON-STIMULATED NERVE AT START	THRESHOLD NON-STIMULATED NERVE AFTER HYDROGEN		CURRENT FOR ACTION POTENTIALS OF EQUAL AMPLITUDE AT START		CURRENT FOR ACTION POTENTIALS OF EQUAL AMPLITUDE AFTER HYDROGEN			
									Stimulated nerve		Non-stimulated nerve	
			Increase			Increase	Stimulated nerve	Non-stimulated nerve		Increase		Increase
			per cent			*per cent*				*per cent*		*per cent*
80	70	156	95	76	96	26	108	84	176	63	106	26
76	60	168	121	106	176	66	92	130	208	126	190	46
96	120	220	129	86	168	93	116	132	292	152	168	27

Threshold in arbitrary units of primary current.

and Hopkins (1926) have shown decremental conduction in nerve does not occur. Field and Brücke (1926) found the refractory period of nerve prolonged in fatigue.

To determine the effect of anoxemia and carbon dioxide on the absolutely refractory period of nerve to stimuli maximal for the smallest fibers, a technique similar to that described by Erlanger, Gasser and Bishop (1927) was employed. Very slight or no differences at all in the absolutely refractory period of the different fiber groups of freshly excised normal nerves was found, thus checking the results of Erlanger, Gasser and Bishop. The results presented in table 4 record the altered absolutely refractory periods of the alpha group alone, those in table 5 the absolutely refractory period of the alpha, beta and gamma fiber groups. Care was taken in our experiments to stimulate slowly (10 times a second) and only to stimulate as often as necessary to obtain required readings. Increasing the

rate up to double the above figure resulted in no appreciable reduction in amplitude of action potential. The increase in the absolutely refractory phase (up to 400 per cent) is still not sufficient to cause interference be-

TABLE 3

MINUTES IN NITROGEN	CURRENT FOR THRESHOLD RESPONSE			CURRENT FOR MAXIMAL RESPONSE			MINUTES IN OXYGEN	CURRENT FOR THRESHOLD RESPONSE			CURRENT FOR MAXIMAL RESPONSE		
Nerve I													
0	82	124	328	116	336	392	0	100	Absent	Absent	164	Absent	Absent
30	84	128	Absent	112	328	Absent	7	118	184	356	184	356	372
60	92	138	Absent	130	248	Absent	37	112	168	316	168	268	420
90	100	Absent	Absent	164	Absent	Absent							
Nerve II													
0	87	144	264	114	280	348	0	222	Absent	Absent	320	Absent	Absent
30	94	148	276	152	222	320	10	188	268	356	268	320	376
120	222	Absent	Absent	320	Absent	Absent	45	140	192	284	196	300	356
							80	136	180	284	164	284	356
Nerve III													
0	100	176	372	192	360	440	0	320	388	460	372	460	520
5	128	252	400	388	432	484	3	276	348	428	332	436	476
10	300	308	460	356	460	520	10	248	348	416	316	400	456
15	320	388	460	372	460	520	35	204	284	372	298	360	336
Nerve IV													
0	80	144	240	112	188	260	0	196	312	400	276	340	440
10	156	260	300	248	308	356	5	156	244	336	244	264	328
15	196	312	400	276	340	440	20	84	144	240	136	232	272
							30	80	136	240	132	232	272

Threshold in arbitrary units of primary current.

tween successive responses whose interval is still 25 times the longest refractory period. Our results showing the effect of stimulation on the threshold of nerve in anoxemia give adequate evidence to permit the conclusion that fatigue was not a serious complicating factor in our experiments.

An analysis of the results indicates that the absolutely refractory period of nerve is prolonged by anoxemia and carbon dioxide. The duration of the absolutely refractory period can be said to vary with the oxygen supply of the nerve. The order of depression as expressed by lengthening of the

TABLE 4

Changes in absolutely refractory period of alpha wave by anoxemia and carbon dioxide; recovery on restoration of oxygen

MATERIAL	THRESHOLD NORMAL NERVE	REFRACTORY PERIOD NORMAL NERVE IN SIGMA	MINUTES IN NITROGEN OR CARBON DIOXIDE	THRESHOLD AFTER NITROGEN	REFRACTORY PERIOD AFTER NITROGEN	MINUTES IN OXYGEN	THRESHOLD AFTER OXYGEN	REFRACTORY PERIOD AFTER OXYGEN
Bullfrog I.....	96	0.87	145 (N_2)	332	3.27	35	136	0.97
Bullfrog II.....	78	0.83	110 (N_2)	120	2.05	65	96	0.83
Bullfrog III.....	80	0.83	240 (N_2)	148	2.38	30	100	0.83
Bullfrog IV.....	88	0.85	10 (CO_2)	120	1.02	20	96	0.90

TABLE 5

Changes in absolutely refractory period of alpha, beta and gamma fiber groups by anoxemia and in recovery, bullfrog sciatic. June 12, 1928

THRESHOLD A GROUP	MINUTES IN NITROGEN	ABSOLUTELY REFRACTORY PERIOD IN SIGMA			CONDUCTION RATE A FIBERS M PER SEC.	REMARKS
78	0	0.83	0.87	1.05	63.64	Readings marked
78	30	0.97	1.05	1.25	54.69	absent represent
90	60	1.18	1.45	2.13	48.35	last reading
100	80	1.35	2.10	Absent	44.36	possible before
110	90	1.70	Absent	Absent	39.94	extinction
120	110	2.05	Absent	Absent	37.23	
	MINUTES IN OXYGEN					
102	8	1.05	1.25	Absent		Recovery period
96	63	0.83	0.87	1.05	59.57	
124	88	0.83	0.87	1.05	51.09	
118	125	0.83	0.87	1.05	56.90	

absolutely refractory period is first in the gamma fibers, then the beta fibers and finally the alpha fibers. *It is significant to note that the ultimate degree of lengthening of refractory period time in all fibers before extinction is approximately equal (graphs III and IV).* In the recovery process the absolutely refractory period again shortens, the degree of shortening being

comparable to the degree of restored irritability. Recovery is most rapid in the alpha fibers, then the beta fibers and lastly the gamma fibers (graph III).[4] The final time for complete recovery has been very nearly equal in all fiber groups with the late fibers requiring a slightly longer period. Again it is pointed out that the time period of depression beyond the stage of loss of reactivity has been longest in these fibers (graphs III and IV). Conduction rate varies inversely with the duration of the absolutely refractory period. In graph II and figure 3 nos. 1, 2, 3, 4 and 5, attention

TABLE 6

Conduction rate changes during depression by anoxemia and carbon dioxide; recovery in oxygen

BULLFROG	NORMAL THRESHOLD	NORMAL CONDUCTION RATE	MEDIUM	MINUTES IN MEDIUM	DEPRESSED THRESHOLD	DEPRESSED CONDUCTION RATE	MINUTES IN OXYGEN	RECOVERY THRESHOLD	RECOVERY CONDUCTION RATE
4	79	44.5	Nitrogen	120	220	35.8	70	132	39.7
2	80	46.7	Nitrogen	96	98	37.2	37	112	39.0
5	84	32.5	Hydrogen	120	188	21.3	180	132	26.5
6	100	45.1	Hydrogen	135	336	28.0	35	172	33.4
7	76	43.9	Carbon dioxide	10	196	29.5	30	140	38.3
8 (June)	76	58.4	Carbon dioxide	11	104	35.9	25	70	48.6
17 (June)	80	61.94 Temp. 24°C.	Nitrogen	240	148	26.85	340	86	59.32
38 (June)	78	63.64 Temp. 24°C.	Nitrogen	100	1,280	37.70	63	96	59.57

Threshold in arbitrary units of primary current.

is directed to the observation that at the commencement of the period of depression when the conduction rate is decreasing and the absolute refractory period increasing it is still possible to find an increase of irritability

[4] Differential depression and recovery of fibers somewhat paralleling those here reported has been found in certain nerves by Gasser and Erlanger (in press) as a result of the application of cocaine. In view of the common assumption that anesthetics interfere with oxidative metabolism and of the known fact that cocaine also prolongs the refractory period of nerve (G. Kato) the natural inference is that the results of Gasser and Erlanger and those here reported have a common factor; and that interference with the oxidative mechanism may be the mode of operation of cocaine.

over normal. This does not occur in all nerves. More usually the conduction rate increases during the first stage of heightened irritability. Opposite in character in this case is the condition noted during the beginning of the recovery stage where in spite of a definite increase in irritability the conduction rate continues for a time to decrease while the absolute

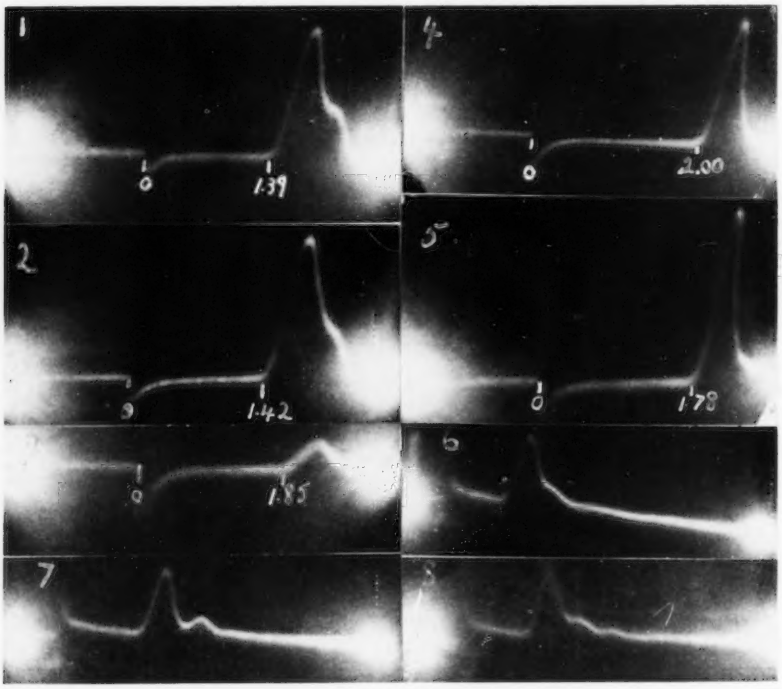


Fig. 3. Films 1, 2, 3, 4 and 5. Conducted action potentials bullfrog sciatic. Note changes in conduction rate occurring with changes in irritability and absolutely refractory period as graphically represented in graph II.

Films 6, 7 and 8. Conducted action potentials bullfrog sciatic. No. 6 normal. No. 7 shows marked separation out of beta wave after 10 minutes in atmosphere of carbon dioxide. No. 8 shows partial return to normal after 20 minutes in oxygen.

refractory period has already begun to decrease (the usual finding is an increase in conduction rate). A discussion of these results is deferred to a later section of the paper.

Changes in the conduction rate with anoxemia, carbon dioxide and lactic acid. Conduction rate is decreased as indicated in table 6. Changes in conduction rate usually go hand in hand with changes in threshold and

duration of the absolute refractory period. A *decrease* in threshold may be coupled with a *decrease* in the duration of the absolutely refractory period in the recovery stage even before an increase in conduction rate takes place, as has been noted in the preceding section.

It is not possible to satisfactorily follow changes in conduction rate of the individual fiber groups, especially the beta group, throughout an ex-

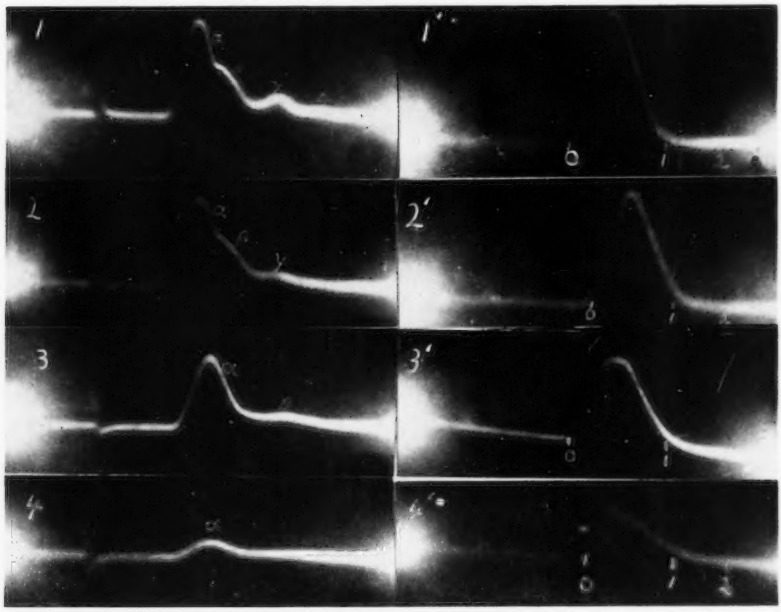


Fig. 4. Films 1, 2, 3, and 4. Conducted action potentials bullfrog sciatic. Illustrate the order of disappearance of alpha, beta, gamma and delta waves by anoxemia. No. 1 normal nerve. No. 2 after 30 minutes in atmosphere of nitrogen, delta wave practically gone. No. 3 after 80 minutes in nitrogen delta and gamma waves gone leaving only lower alpha and beta waves. No. 4 after 120 minutes in nitrogen only lower alpha waves remains.

Films 1′, 2′, 3′ and 4′ correspond to above films taken near the stimulus. Note depression in amplitude and unaltered duration of action potential when slight allowance is made for spread due to differences in connection rate of alpha and beta fiber groups, this spread indicated by widening of crests.

periment because of changes in form of the conducted action potential. However, it can be stated that if allowance is made for fiber group rearrangements the evidence indicates that slowing is first and most rapid in the delta fibers, and least rapid in alpha fibers. This is consistent with the definitely determined changes in the rate of increase of their respective threshold and the ultimate order of depression of their amplitude.

There was noted a tendency to diphasicity of the recorded action potential increasing with the degree of depression in those preparations having a considerable distance between the killed end and the ground electrode. This is considered the result of a showing of the conduction rate of the nerve fibers. This has an effect similar to increasing the distance between the killed end and the ground electrode. It has been shown by Bishop, Erlanger and Gasser (1926, loc. cit.) that the killed margin serves as a lead for a depressed second phase which tends to oppose the "monophasic" potential and renders it distinctly diphasic with sufficient separation of the effective leads. The diphasicity in our preparations could be eliminated by killing close up to the ground electrode.

During recovery conduction rate increases. It increases most rapidly in the alpha fibers and least rapidly in the gamma fibers. It does not always increase to the previous normal even in those nerves showing a return to a threshold lower than the normal. A partial recovery of conduction rate may be associated with incomplete recovery of the absolutely refractory period time, and might indicate a change in vitality, but it may also be dependent on changes in the physical reactance of the nerve.

Changes in the form of the conducted action potential in anoxemia and carbon dioxide. The area and amplitude of the conducted action potential taken as a whole are gradually reduced to extinction (fig. 4, nos. 1, 2, 3 and 4).

The action potentials of the delta and gamma groups are first depressed to such a state, then the beta group and lastly, the alpha group. Separation between the fiber groups may seem quite pronounced in the early stages of depression so that the falling phase of the alpha or beta waves may almost reach the base line before the start of the next wave (fig. 3, nos. 6, 7 and 8). This may be accounted for by differential changes in the conduction rates of the fiber groups. It is interesting to note that the waves separate out more distinctly some time after excision than when fresh; evidence of differential change of conduction rates with earliest deterioration of smallest fibers. Such differential depression has the same effect on the form as recording at a greater distance.

The duration of the conducted compound action potential is first increased due to a slowing of conduction rate of the fiber groups. With the elimination first of the delta and gamma and then of the beta wave shortening occurs. In many experiments in which changes in the action potential on conduction were followed, the action potential was also recorded at the stimulus. Always when an action potential was elicited at the stimulus there was also a conducted action potential. There was never any evidence to support the theory of conduction with a decrement.

In the recovery process the amplitude of the conducted action poten-

tial increases (fig. 2, nos. 10, 12 and 14). This increase may continue to a point beyond the original normal. The total duration of the conducted action potential first increases with the return of the beta and gamma groups and then decreases. This decrease may again proceed to a point beyond the normal in those nerves showing an abnormal decrease in threshold and increase in amplitude of action potential at the stimulus (fig. 2, nos. 9, 11, 13, 10 and 14). Often noticeable is the marked separation of the individual waves in the early stages of recovery. In the late stages the dissociation of the fiber groups may be less marked than in the normal (fig. 2, nos. 10 and 14). Recovery in amplitude is most rapid in the alpha group and least rapid in the gamma group. The rate of increase in amplitude is remarkably rapid as compared to its decrease in the process of depression.

Changes in the form of the action potential at the stimulus due to anoxemia, carbon dioxide and lactic acid. In a previous paper from this laboratory the effect of the escape on the form of the action potential at the stimulus has been analyzed by Bishop (1927). As the strength of the stimulus is increased the apparent crest time is decreased because of the effect of summation of the escape with the action potential. A decrease also results from the establishment of a more nearly equal point of stimulation of all fibers in relation to the ground electrode with such stimuli. In our present work it was not found practicable to eliminate all distortion due to escape or to employ a type of stimulation free from the defects of the electrical one. During depression of anoxemia, carbon dioxide and lactic acid, the use of constantly increasing strengths of stimulation are necessitated. Consequently progressive distortion of the position of the crest becomes inevitable.

Elsewhere we (1928) have described the origin of responses single or repetitive under the anode with short induction shocks. The presence of anodal responses following the cathodal response causes the crest of the double action potential to be later than that of the single action potential arising at the cathode (fig. 1, nos. 1 and 4; fig. 2, nos. 1 and 2). (With maximal stimuli the cathode response in all fibers blocks a single anodal response and thus may shorten the crest time.) The facts are of importance because of changes in the responsiveness of nerve to stimulation under the anode in anoxemia.

In anoxemia, or as a result of carbon dioxide and lactic acid, it is found that the amplitude of the action potential at the stimulus is depressed, ultimately to extinction (figs. 1 and 2).

By shunting across from grid to ground action potentials of equal amplitude can be obtained. When submaximal action potentials recorded during depression are made of equal amplitude and compared there is seen a shortening of the crest time and the narrowing of the wave itself

(fig. 1, nos. 1 and 3, 5 and 6, 9 and 10). For maximal responses no definite changes in crest time of form are discernible. Likewise for definitely submaximal action potentials recorded during depression there may be a shortening of the falling phase which is not present in maximal action potentials. The loss of any response under the anode with a resultant shortening of the crest time (fig. 2, no. 6) occurs early in anoxemia, and is not the explanation of the above shortening.

In all our experiments, the escape, cathodal in direction at the start of the experiment, soon became anodal and of longer duration (fig. 1, nos. 5 and 6). This change occurred with the decrease in threshold at the commencement of depression referred to earlier in the paper. It is apparently an expression of a change in the physical reactance of the preparation.

To appreciate the significance of changes in the form of the falling phase of the action potential it is necessary first to consider variations which may occur in the normal nerve. With weaker stimuli in irritable nerves the spread of stimulating current through the nerve is such as to cause the stimulation of the individual fibers at different points between the effective anode and cathode. This results in a widening and lowering of the crest and a lengthening of the falling phase of the submaximal action potential (10 to 20 per cent) as compared with a maximal response (fig. 2, nos. 5 and 6). The degree of spread seems greater in more irritable nerves. In summer frog nerves it is frequently absent or very transient disapprearing rapidly even in the presence of oxygen. It becomes less marked and disappears entirely in nerves preserved in Ringer's solution. The shortening of the falling phase as the potential becomes maximal can be accounted for by assuming that all fibers are stimulated nearer to the same cross section in relation to the recording electrode with stronger stimuli.

It was invariably found that during anoxemia or exposure to carbon dioxide and lactic acid, the durations of the falling phases of successive action potentials recorded at equal amplitudes, but definitely below the maximum, shorten rapidly to the degree to which the normal action potential can be shortened on increasing the strength of stimulus to maximum (fig. 2, nos. 1 to 8). The duration of the *maximal* action potential throughout the period of depression remains practically unaltered (fig. 2 and fig. 4). It might be added that experiments with short galvanic stimuli revealed that either by increasing the strength of stimulus or by shortening its duration the falling phase of the action potential at the stimulus can be shortened to a fixed minimum. If the duration of stimulus is shortened beyond this point response fails. This shortest duration of the falling phase of the action potential is also the duration to which it is reduced by depression with anoxemia and carbon dioxide and lactic acid.

In the course of recovery the amplitude of the action potential at the

stimulus increases, occasionally to a point beyond the maximal for the normal nerve (fig. 2, nos. 9, 11 and 13). In such cases there is also an increase in total area. The strength of stimulus required for a maximal response may be less than in the previous normal. The crest time lengthens as the strength of stimulus required decreases but the duration of the falling phase remains practically unchanged indicating that the fibers continue to be stimulated at more nearly the same cross section point of the revived nerve than in the normal.

The normal action potential wave in oscillograph records does not return promptly to the original base line, but exhibits a long "tail" of potential lasting for many sigma. Bishop (1927, loc. cit.) interpreted this as "not a part of the excitation phenomenon proper, but a sign of some recovery or restoration process," to which the excitation potential returns as to a new base line. Davis, Pascual and Rice (1928) have recently offered a similar interpretation, based upon string galvanometer records in which the long lasting potentials are more evident. In the present experiments only the main wave is dealt with. The end of this wave, since it approaches a base line asymptotically, is impossible to determine. However the falling phase inflects sharply near its end, and an approximate terminus may be assigned. Whenever possible, two records to be compared were made at the same speed and shunted to the same amplitude, when the whole duration of the falling phases could be compared by superposition. When changes occurring in recovery are considered together with those occurring in the process of depression it becomes more and more evident that all changes in the form of the submaximal action potential recorded near the stimulus can be accounted for by assuming that stimulation in all fibers occurs more nearly at the same distance from the ground electrode. The alteration in nerve responsible for this change seems an irreversible one. For reasons given the form of the action potential for a maximal response is considered more nearly that of the individual axon than is that for the submaximal. In final analysis therefore it can be said no definite evidence of a change in the form of the main axon action potential wave at the stimulus is to be inferred. Changes in the form of the long-lasting retention have not been followed.

Discussion. *Differential depression of functions of nerve.* Since amplitude of action potential, conduction rate, absolutely refractory period and threshold do not vary together under depression of nerve function it follows that there must be at least two more or less independent variables underlying them. One of these variables *is obviously the available oxygen.* The absolutely refractory period varies consistently with this, that is, upon reduction of oxygen tension the absolutely refractory period lengthens at once and vice versa for an increase of oxygen tension. There is never a change in the opposite direction so often ob-

served in the other properties of nerve. *We may therefore look upon refractoriness as a simple measure of one aspect of function.* The other properties of nerve then are affected, not only by this factor, but by something else which determines how easily the nerve may be stimulated, when it can be stimulated at all.

Such a factor might be the physical reactance of the nerve circuit (polarizability, etc.) which might change with the accumulation of acid metabolites, etc. Evidence of such a change is the initial decrease of threshold often occurring when other functions of the nerve's reactivity are depressed, and the change in the artefact due to the shock under anoxemia. The changed form of the submaximal action potential wave (shortening) during and after anoxemia, usually associated with an increased threshold, may also be assignable in part to this factor. Such a change in resistance might further affect conduction rate on a physicochemical basis such as that discussed by Lillie (1916) so that at the beginning of anoxemia the decrease in resistance might more than compensate for changes in the metabolic state of nerve indicated by refractoriness, and conduction rate would be increased. If the resistance change did not compensate for such depression in the metabolic state, conduction would be decreased as is always the case as anoxemia proceeds further.

It is much more difficult to account for the occasional temporary decrease in conduction rate witnessed at the beginning of recovery in the presence of a *decrease* in threshold and a *decrease* in absolutely refractory period time. That the reactance of the nerve to a stimulating current does not seem to be markedly altered on the restoration of oxygen follows from our observation that the stimulating current is effective at more nearly the same cross section point in all fibers of the recovered nerve than in the normal. It is also possible that other factors are involved in conduction than those evidenced in irritability and refractoriness. The change in physical reactance assumed to occur at the beginning of anoxemia might account for the increase in amplitude in action potential over the normal observed at this time. This increase in amplitude is, as noted above, also associated with a change in the character of the escape. In the altered nerve the positive phase of the artefact becomes prominent and of much longer duration. Such a form of escape had been previously noted by Bishop, Erlanger and Gasser (1926). It is apparently indicative of an increased polarization ability under the anode. Stimulation however still occurs under the cathode. The escape is possibly the result of a reversal of polarization from anode to cathode. There was no evidence to indicate stimulation under the anode itself, in fact all anodal responses were immediately abolished with the advent of this form of escape. The phenomenon may bear a very definite relationship to the changes in polar stimulation in anoxemia reported by Thörner (1924).

Factors affecting nerve function. As a tentative scheme, these factors may be analyzed as follows.

1. Physical reactivity—permeability, polarizability, i.e., the ease with which the stimulus may reach the critical point of nerve, or the resultant excitation current flow to the recording system.

2. Chemical reactivity—the available nerve material and its chemical state, affecting the ease of setting up a reaction and the intensity of its result.

Threshold and amplitude of recorded potential, possibly conduction rate, should depend on both these factors. The refractory period might depend only upon the second. A distinction might be drawn between irritability, as a function of reactivity, not precisely measurable as such, and threshold, the measured stimulus, as further affected by the physical conditions determining the efficiency of the applied energy.

The axon potential as an index of the functional state of nerve. That the axon action potential as recorded is not always a true indicator of the nerve's metabolic state is emphasized by our results. Its form except for amplitude has been shown to remain unaltered in anoxemia, in the depression of carbon dioxide and lactic acid. It has been shown that the amplitude of the action potential may actually increase in anoxemia when the absolutely refractory period time is increasing and conduction rate is decreasing. After practically complete recovery as indicated by a normal refractory period the amplitude may be greater than that of the nerve before anoxemia at a time when the conduction rate is less than the normal.

Absolute refractory phase of fibers of different sizes. Previous investigations of Erlanger, Gasser and Bishop (see papers from this laboratory) tended to show that fibers of different sizes conduct processes that are essentially similar. The differential effects displayed are interpretable as functions of the relationship between the surface area and volume of the fibers. Conduction and area of potential curves are dependent on fiber size (Gasser and Erlanger, 1927) and threshold also varies approximately inversely as diameter (Bishop and Heinbecker, 1928). The present results show that the absolutely refractory periods of all fibers in fresh nerves are practically the same, confirming Erlanger, Gasser and Bishop (loc. cit.) and that all fibers of freshly excised nerve reach approximately the same degree of refractoriness before extinction under anoxemic conditions. That the refractory period times of nerves do not remain identical on standing in air or even in Ringer's solution has been repeatedly demonstrated in our experiments. Such nerves show the same type of differential depression as exhibited in anoxemia or in carbon dioxide, in which the gamma fiber absolute refractory period time becomes longer than that of the beta fiber and the latter longer than that of the alpha fiber. Differences in the refractory phases of the alpha, beta and gamma fibers of normal nerves

originally reported from this laboratory may have been due, not to inaccurate measurements or to fundamental differences or variability of the fiber processes but to a differential deterioration after excision. Such differential deterioration as well as that found in anoxemia and in the presence of high concentrations of carbon dioxide might result from the differences in the volume-area ratio of the fibers. This ratio should govern 1, the rate of change in concentration or diffusion of gases, and 2, the amount of metabolic reserve underlying the ability of the nerve to resist deterioration. Judging from the fact that depression in lack of oxygen is too slow a process to be assigned to simple diffusion of physically dissolved oxygen, the latter of the above factors seems to be the important one.

Mechanism of depression in anoxemia and in the presence of abnormal concentration of acid. It is natural to suppose that lactic and carbonic acids interfere with normal metabolism due to the H ion concentration effect. Lack of oxygen might similarly lead to an accumulation of non-diffusible acids. However, an increased H ion concentration in general is apt to depress oxidation itself, a decrease to favor it. This is especially true in the oxidation of carbohydrates. We might expect therefore that lack of oxygen would depress the nerve in two ways, first by depriving it of one reactant in the chemical process, and second, by depressing, due to acidity, the reaction that might still be going on. CO_2 could have the latter effect without the former. Thus, the difference between the curves of depression under nitrogen and under CO_2 may be assigned to the H ion alone, the recovery in the absence of gaseous oxygen from the effects of CO_2 being a recovery only to the corresponding curve for depression by lack of oxygen itself.

Such a recovery indicates an oxygen reserve remaining after a considerable exposure to oxygen free CO_2, (as much as an hour's exposure) a reserve that persists for too long a time to be explained as free gaseous oxygen.

Investigators like Fröhlich (1904) have stressed the idea of an oxygen reserve accounting for the prolonged activity of nerve in anoxemia. Gottschalk (1921) attempted to discredit its importance. Our own experiments are consistent with the ideas of Fröhlich. The fact that partial restoration can be accomplished by surrounding a nerve already depressed by carbon dioxide with pure nitrogen hardly permits of any other interpretation. It is most probably the increase of hydrogen ion concentration which inhibits the utilization of oxygen. The degree of restoration in nitrogen varies with the time of carbon dioxide exposure, simply indicating that the CO_2 is not only inhibitory to oxidation, but is also depressive as an oxygen free gas. Since stimulation during anoxemia hastens such depression, the oxygen reserve presumably is depleted both by physiological response and even by the resting metabolism without stimulation.

The action of acid produced in anoxemia may be involved in the ability to bring about a partial temporary restoration with oxygen free Ringer's immediately after complete depression by anoxemia of 2 or 3 hours' duration. The washing might remove some of the acid products of metabolism and permit the utilization of the remaining stored oxygen. A similar significance is attached to the observation that if a nerve whose activity is abolished by anoxemia and then allowed to recover even to normal activity for a short period, it requires a much briefer period of anoxemia for a corresponding degree of depression than it did the first time. The prolongation of functional activity in anoxemia by washing with Ringer's, and also the greater ability of nerves to recover to normal in oxygen when washed are facts needing no further comment.

SUMMARY

The effect of anoxemia, carbon dioxide and lactic acid on nerve is, in general, to raise the threshold, to lengthen the absolutely refractory period, to decrease conductivity and the amplitude of action potential. These effects are reversed in recovery induced by oxygen and washing with isotonic Ringer's solution.

In general certain coördinative relationships exist between threshold, refractoriness, conductivity and action potential. Exceptions to the general rule occur and their origin may possibly be attributed to changes in the physical reactance of nerve during depression and recovery. Experimental evidence presented establishes the independent variation of certain of these properties of nerve and indicates at least a partial difference in their mechanism.

Differential changes in threshold, refractoriness, and conductivity in the different fiber groups are recorded. Depression is most rapid in the smallest or delta fibers and least rapid in the largest or alpha fibers. This differential depression is presumably the result not of fundamental differences or variabilities in the individual fiber group but the physical consequence of the volume area ratio of the fibers. Recovery is most rapid in the large fibers.

Further proof of the fundamental similarity of the fiber groups is found in the fact that they acquire the same degree of increase of refractory period before becoming unirritable. Refractoriness apparently varies with the available oxygen supply and is considered a true index of the nerve's metabolic state.

The action potential recorded near the stimulus is depressed in amplitude. For action potentials definitely below the maximal there occurs a shortening of the crest time and a shortening of the falling phase. Such changes do not occur when maximal action potentials only are compared. For reasons discussed the maximal action potential is considered to ap-

proximate more closely to that of the axon action potential than does the submaximal. Consequently no change in the actual form of the axon action potential is considered to occur. "Retention of negativity" has been disregarded as probably not a part of the response process proper.

The changes in recorded form including amplitude of the recorded action potential may in part be assigned to changes in physical reactance of the nerve. The potential then is not a precise index of the nerve's metabolic state.

Changes in form of the conducted action potential are recorded. They are chiefly due to differential depression of potentials and of conductivity of the fiber groups.

Evidence is presented to indicate that the detrimental effect of carbon dioxide is primarily due to acidity interfering with the use of oxygen and secondarily to the loss of oxygen resulting from changes in partial pressure. The detrimental effect of anoxemia on the other hand is principally due to a loss of oxygen physically dissolved or chemically combined. It must in part be due also to changes dependent upon the accumulation of acid products of metabolism.

Action potentials recorded practically simultaneously at the stimulus and after conduction showed that conduction with a decrement in nerves depressed by anoxemia and carbon dioxide does not occur.

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SOME ASPECTS OF THE PHYSIOLOGY OF ANIMALS SURVIVING COMPLETE EXCLUSION OF SYMPATHETIC NERVE IMPULSES

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The approach to the present research was described in a brief report by Cannon, Lewis and Britton (1927) on the "dispensability of the sympathetic division of the autonomic system." Our search for the disturbing factor which accelerated the heart after known nervous and humoral accelerators had been eliminated involved ultimately removal of almost all of the sympathetic division (see Cannon, Lewis and Britton, 1926). The apparent absence of serious effects of the operation (in cats) led us to remove the sympathetic system—i.e., the two chains of ganglia—from the superior cervical ganglia to the two united ganglia just below the rim of the pelvis. As stated in the earlier report, animals thus operated upon have lived in the quiet conditions of the laboratory for many months, apparently in perfect health. They manifest a number of interesting phenomena which will be described in this paper.

PROCEDURE. *Operation A.* In the earlier instances the sympathetic chains were removed in parts, corresponding roughly to the neck, chest and abdomen. Usually in our first operations the two cervical sympathetic cords from above the superior to below the inferior ganglia were isolated and excised in a single operation. Next the abdominal strands were taken out from the diaphragm to the pelvis, together with the splanchnic nerves on one side. Then the thoracic sympathetic chain on each side was lifted or cut away from its connections, from the stellate ganglion to a point just above the diaphragm, and withdrawn. And finally the remaining splanchnic nerves, which were traced into the thorax, and also the semilunar ganglia (or "solar ganglion," as Langley called the mass) were removed. After each step the animal was restored to full vigor before the next step was taken. The defect of this method lay in the possibility of leaving small bits of the sympathetic strands, especially in the lower part of the thorax, because cicatrization obscured the exact point to which the previous operation had extended. Nevertheless, as tested by erection of the hairs on ex-

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posure to cold or during excitement, complete elimination of sympathetic effects was obtained (see fig. 3).

Operation B. In later operations we were content to eliminate all influence of the sympathetic system in the organism by removing completely at one operation the entire chain from the stellate to the pelvic ganglia first on one side and later on the other. Through an opening between the fifth and sixth ribs the thoracic chain was dissected free from above the stellate to a point as low as could be reached; there the loosened mass was left while the chest wall was closed. The process was continued through an opening between the ninth and tenth ribs, and the chain, drawn downward,

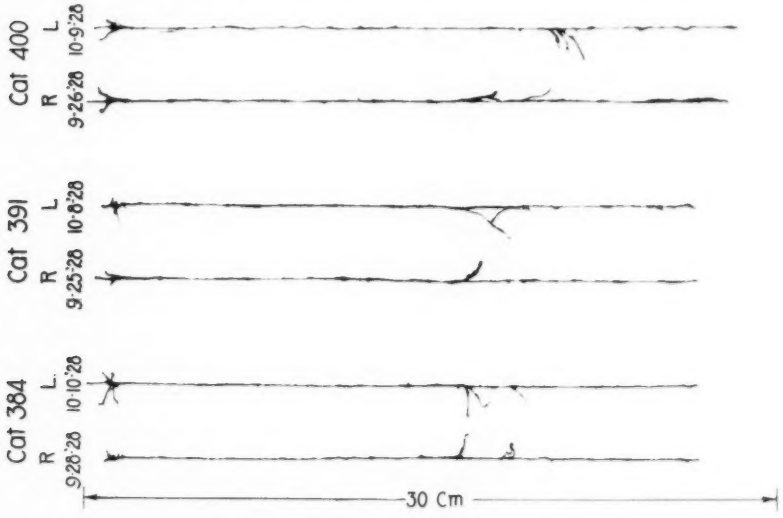


Fig. 1. Examples of thoracic and abdominal sympathetic chains removed intact. They were dried and with collodion were mounted on the card before being photographed.

was left lying just above the diaphragm. Finally through the abdominal midline the thoracic chain was pulled past the diaphragm, the splanchnic nerves were severed, and, after the abdominal portion had been resected well into the pelvis, the entire chain was delivered intact. The time required for a unilateral sympathectomy usually varied between 60 and 70 minutes. The post-operative course was uneventful except in rare instances of septic infection. After two or three weeks the animal was in vigorous condition again and then the other chain was removed in like manner. In the cat this method of sympathectomy was repeatedly proved quite feasible (see fig. 1).

When sympathectomy was performed in the dog or monkey the two abdominal chains were usually removed first from pelvis to diaphragm and the splanchnics severed on one side; next the thoracic chain was taken out on the side deprived of splanchnic connections; and finally the remaining thoracic chain was wholly extirpated. By this procedure a maximal period intervened between the severance of the two major sets of splanchnic nerves which largely control vascular tone in the abdominal viscera.

Whether operation A or B was performed the method of opening and closing the chest wall was that employed by Cannon, Lewis and Britton (1926) in denervating the heart. Milk (but no meat) was given the day before operation, and the post-operative care and feeding were the same as described in the earlier account. The most efficient and least disturbing method of sympathectomy, which was especially applicable to cats, consisted of complete unilateral removal of one chain from stellate to pelvis and at the same time removal of the other abdominal chain, except the major splanchnic. Subsequent complete excision of the remaining thoracic chain through two chest openings assures also section of the major splanchnic trunk. Thus there are only two operative disturbances to be recovered from.

Fig. 2. Photograph of a cat from which the right thoracic and abdominal sympathetic chain had been removed. By cooling the animal the still innervated hairs were made to stand erect. The right cervical ganglia were disconnected from the central nervous system but still retained connection with pilomotor muscles of the right side of the head. Note that the hairs of that region are not standing.

In the operation B, described above, in which the cervical chains are left in place, the question may arise as to the justification of the expression, "complete exclusion of sympathetic impulses." Because all connections between the sympathetic and the central nervous system lie between the eighth cervical spinal nerve and the fourth lumbar we necessarily broke the entire series of connecting fibres when we extirpated the chains from the stellate to the pelvic ganglia. Furthermore, by taking away the ganglia in the chains we deprived the post-ganglionic fibres of their nutritive centers, and these fibres must therefore degenerate. There were left, to be sure, the cervical sympathetic and the semilunar ganglia, as well as small ganglia near the root of the tail, but they were no longer connected with the central nervous system and consequently could not serve to carry messages sent forth from the brain (see fig. 2). Indeed, no difference was observed

between the animals in which these isolated ganglia were left in place and the animals from which they were removed. Morphologically, therefore, the second type of operation was not a complete sympathectomy but rather a total "sympathotomy;" physiologically, however, it was equivalent to a complete sympathectomy.

RESULTS. *Continued existence of sympathectomized animals.* About nine years ago Meltzer (1920) reported that simultaneous removal of the superior cervical ganglia from 28 rabbits resulted in a more or less prompt death of 25, with pulmonary lesions prominent at the autopsy. Two cats similarly operated on died in less than two days and examination showed again pathology in the lungs. In a cat which survived, the operation was proved to have been incomplete. From this evidence Meltzer was inclined to attribute the fatal outcome solely to the ganglionectomy, and he definitely stated that the "superior cervical ganglia contain a principle which is essential for the maintenance of life." Montgomery (1923) found, however, that bilateral superior cervical sympathetic ganglionectomy, in rats, cats and rabbits, was quite compatible with life. A similar inference may be drawn from the absence of serious results after the Jaboulay operation, a bilateral removal of the ganglia in one operation, which has been performed on man for the treatment of glaucoma, exophthalmic goitre and other abnormal conditions. In our experience we have seen effects which might well account for the results described by Meltzer. Occasionally severe and sometimes fatal symptoms appear in the cat when both cervical sympathetic chains are removed simultaneously. There is marked congestion of the nasal mucosa and a heavy mucous exudate, in all probability secondary to loss of vascular tone from the denervation. The animal necessarily breathes through the mouth and it seems likely that a pulmonary infection follows because the natural protection provided by the nasal passage is lacking. If the two cervical sympathetic chains are removed separately, with an interval of eight or ten days, no such serious results follow. By the time of the second operation sufficient peripheral tone seems to have developed in the vessels of the side first denervated to enable the animal to breathe normally. Meltzer's intimation that the fatal effects of superior cervical ganglionectomy might be due to removal of some important internal secretion associated with the ganglia receives no support in our observations.

Spadolini (1926) has declared that complete and extensive extirpation of the mesenteric nerves of the cat is incompatible with continued existence. General depression, grave wasting, lowering of body temperature, and lessened frequency of respiration appear, and death follows in from three days to about a month. He also noted dystrophy and ulcerations of the skin, clonic, fibrillary and at times tetanic contractions of various groups of muscles, and a diminished appetite or total anorexia. Salivation was

abundant, vomiting occurred, and also constipation alternating with crises of sanguineous diarrhea. *Post mortem* he always found severe lesions of the gastro-intestinal tract, especially of the small intestine, characterized by necrosis and sloughing of the mucosa, and also widespread lesions in other structures. Spadolini concluded that the mesenteric nerves condition the integrity of the intestinal epithelium and thereby regulate the processes of selective absorption regarded as the normal function of that structure. Although we have removed the abdominal sympathetic chains, including the splanchnic nerves, in more than one hundred cats, and although we have also removed from many of them the semilunar ganglia (see figs. 3 and 5) and have even stripped the nerve strands from the celiac axis and the superior mesenteric artery, thereby destroying strictly "mesenteric nerves," we have never seen any serious symptoms which could be ascribed to the procedure. After the temporary set-back due to the operation, our sympathectomized cats ate heartily and gained weight. Nothing abnormal has been observed in the activities of the digestive canal. From our experience the results reported by Spadolini are difficult to understand. Some of the primary disturbances of the intestine which he observed resemble closely those produced by interference with the blood supply. It is possible that in his mode of operating the circulation of the alimentary tract was seriously upset.

Abel (1926) has protested against the conclusion that chromophil tissue is not essential to life—a conclusion based on the apparent innocuousness of ablating one adrenal gland and demedullating the other—for, he declares, "it is quite impossible to extirpate every trace of medullary or chromophil tissue, since tissue of this character is found not only in the accessory suprarenals but also, to a small extent, in every one of the sympathetic ganglia located in the thoracic and abdominal cavities." And since glands of internal secretion have a large "factor of safety," i.e., a small remnant can perform the functions of the whole, "it has not been proved with certainty that epinephrine is not essential to the continuance of life." As stated above, however, we have removed the thoracic and abdominal sympathetic ganglia and thereby have been able to show that even with the adrenal glands left undisturbed not enough adrenin is secreted during excitement and vigorous struggle to cause any noteworthy acceleration of the denervated heart. No more exacting test than that could be applied, for, as Anrep and Daly (1925) have shown, the cat heart is sensitive to adrenin in the dilution of 1 part in 1,400,000,000 parts of blood. But we have gone further. We have removed (from cat 286, a castrated male) both sympathetic strands from the superior cervical to the pelvic ganglia (by operation A), we have almost entirely extirpated the hypophysis, we have dissected out the semilunar ganglia, and we have excised the right adrenal gland and sucked and scraped away the left adrenal medulla. With due

care to maintain his well-being these operations were done in succession during the period between April 21 and October 5, 1927.

He remained in the laboratory for eleven months thereafter, in excellent condition, eating normally and performing his obvious functions like a normal animal. After the last operation (Oct. 5) his weight was 3 kg. instead of 3.4 kg. (the weight in April) but during the next eleven months it increased to 4.5 kg. On September 14, 1928, when the cat was in very good physical health he was killed with ether for purposes of examination. At autopsy about one-third of the posterior lobe of the hypophysis was found. No trace of the sympathetic chains was discovered in neck, chest or abdomen; sections of suspicious filaments in the lower thorax proved them to be fibrous tissue. Examination of the left adrenal, histological and chemical (by the chromate test), proved no medullary cells present. The chromate test applied to the abdominal vessels between the kidneys, to reveal the presence of accessory chromophil ribbons and threads, was negative. From all these observations we conclude that the chromophil tissue, whether in the adrenal medulla or in sympathetic ganglia, is not required for continued existence.

In general we infer from our experiments that no part of the sympathetic system is essential for life. We have operated on cats and dogs as horizontal animals, and on monkeys as animals taking a vertical posture, and have watched them for varying periods thereafter. Our experience with monkeys has been limited thus far to six animals. All survived in excellent condition complete bilateral abdominal and left thoracic sympathectomy; they ate well and had unimpaired agility; unfortunately one died at the start of the final operation because of difficulty with artificial respiration. Three of the other five underwent final removal of the right thoracic sympathetic chain and section of the right splanchnic; they had no difficulty in taking the vertical posture—sitting up and eating bananas and carrots or jumping about the cage in the usual manner. One of them later contracted pneumonia and died. The remaining two have continued healthy and active—one of them since March 5, the second since April 5, 1929. These animals definitely show the compatibility of the upright position with partial or complete sympathectomy. The dog deprived of sympathetic control of its organs is in general appearance indistinguishable from the normal dog. Cats we have kept in the laboratory in some instances for many months, in other instances for more than a year, after the last remnant of the sympathetic had been extirpated. One such animal (107) from which the abdominal sympathetic chains were removed April 8, 1926, the cervical chains May 11, 1926, the thoracic sympathetic to the 11th and 12th ribs February 4, 1927, the splanchnics on both sides and the semilunar ganglia March 1, 1927, and some short pieces from the lower chest October 10, 1927, has lived in the

laboratory as a pet during the entire period and now (May 8, 1929) is to all outward appearances healthy and vigorous. From all this evidence it should be clear that the sympathetic division of the autonomic system is not indispensable—that it is not necessary for survival under laboratory conditions.

Sympathectomy in relation to growth and development. We have removed the sympathetic chain from one side in two kittens six to eight weeks old. The animals grew rapidly and were normally active and playful. Their gait was natural,—there were no signs of any change of muscular function on the sympathectomized side. One of the kittens died from a respiratory infection four months after the operation and the other died after a post-operative period of five months. Each had doubled its weight since the unilateral sympathectomy. At autopsy careful comparison was made of organs on the two sides of the body.

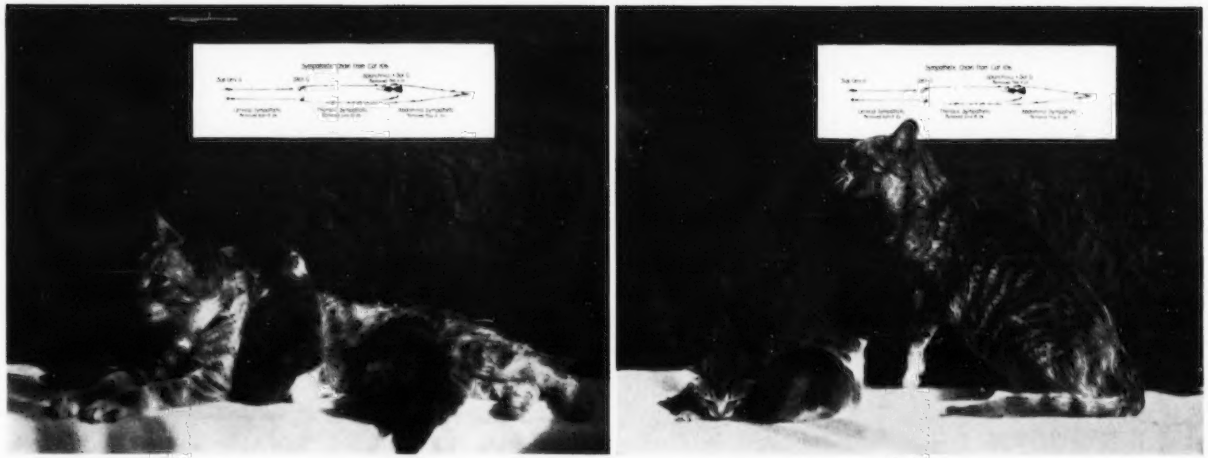
TABLE 1

Weights of bilaterally symmetrical organs of cats previously sympathectomized on one side

	CASE A (4 MONTHS)		CASE B (5 MONTHS)	
	Right (sympathectomy)	Left	Right	Left (sympathectomy)
	grams	*grams*	*grams*	*grams*
Weight of kidneys	9.0	8.7	7.9	8.0
Weight of adrenals	0.20	0.20	0.25	0.24
Weight of thyroids			0.09	0.08
Weight of femurs	7.75	7.61	6.41	6.22
Weight of humeri	5.95	6.51	5.10	5.23

As the figures in table 1 show, there was no consistent or significant difference between these structures on the two sides. Measurement of the length of the femurs and humeri likewise revealed no difference—they were within a millimeter of being the same length on the two sides. These results are in accord with the observations of Wright and Harris (1929) that sympathectomy on one side in kittens causes within four months no change in the growth of the long bones as compared with the normal side. We noted, furthermore, that the distribution of fat was indistinguishably the same on the right and left halves of the body. Indeed, we have found no evidence that the sympathetic division of the autonomic system plays an important part in the growth and development of the organism.

Sympathectomy in relation to the female functions in reproduction. We have no evidence with regard to the ability of the male cat to take part in reproduction after sympathectomy. One of our sympathectomized female cats (106), however, conceived when the splanchnics were the only sym-



A B

Fig. 3. Photographs of cat 106; her sympathetic ganglionated strands—cervical, thoracic and abdominal (including the semilunar ganglia)—mounted on the card above; and also her kittens born after she was completely sympathectomized.

A. One of the kittens nursing, the other playing on the mother's back.

B. A barking dog at the left has roused her to protect her kittens. Note no erection of the hairs on her neck, back, or tail.

pathetic trunks which remained, and six weeks after the splanchnics were extirpated she gave birth to two normal kittens. She nursed not only these two offspring, but also other kittens born at about the same time. When approached by an aggressive dog she reacted sharply to his presence in such manner as to protect her young (see fig. 3). Later one of the kittens had to be killed because of an injury, but the other was brought to the stage of independent nutrition by the sympathectomized mother, and developed into a normal adult cat.

The results noted in the instance just described are in accord with those obtained in this laboratory by Sweet and Thorp (1929). They found that removal of the lower abdominal sympathetic chains in rats had only a temporary effect on the routine recurrence of the oestrous cycle. The observations on cat 106 indicate not only that the phenomena of oestrus are not dependent on sympathetic impulses, but also that the processes of birth and lactation can go on without coöperation of the sympathetic system.

Emotional reactions in relation to sympathectomy. As already noted, we commonly tested the completeness of the operation for sympathectomy by exposing the cat to a barking dog and observing whether there was total absence of erection of the hairs. It was by this means that we discovered that when the thoracic sympathetic chain is taken out by itself, instead of being removed in connection with its abdominal continuation, a short bit is likely to be left just above the diaphragm. Under such circumstances a small tuft of hair rises, during excitement, in the lower midpart of the back—a phenomenon which disappears after the remnants have been removed.

Besides erection of hairs a characteristic feature of an intense emotional response is an increase of blood sugar. The now well-known emotional hyperglycemia may be so great as to result in a glycosuria (see Cannon, 1915). It was a matter of some interest to know how sympathectomy would affect the emotional glycemic reaction. To answer this question observations were made on normal control and on sympathectomized cats under the same conditions and with the same technique. The animals were deprived of food for fifteen hours previous to the experiments. They were then brought to the laboratory. While a cat was resting quietly in the lap of an attendant, the ear, from which the hair had been previously removed with barium sulphide, was quickly nicked over the main vein by means of a sharp blade. It produced no discomfort or excitement. About 1 cc. of blood was collected in a test tube where coagulation was prevented by means of a small amount of lithium oxalate. For the sugar test the Baumann-Isaacson (1922) modification of the Folin-Wu method (1920) was employed, requiring only 0.4 cc. of blood. Each sample was tested twice. Care was taken to use fresh standards, for the standards weaken in

the course of an hour or more. After the blood had been obtained under quiet conditions the animal was fastened to a holder (see Cannon, Shohl and Wright, 1911), where it usually became excited because of the restraint and struggled vigorously. If one or two legs were left unfastened the struggle was often more energetic than if all four were held tight. When the excitement had lasted for some time (varying from 2 or 3 to 10 minutes)

TABLE 2

Results of tests of blood sugar in normal cats (100, 111, and 113) and in sympathectomized cats (104 and 107) before and after excitement and struggle

(For 104, see fig. 5; for 107, see description, page 89)

NUMBER	DATE (1927)	WEIGHT	BLOOD SUGAR		EXCITEMENT	PERCENTAGE CHANGE
			Quiet	Excited		
Control cats						
		kg.	*mgm. per cent*	*mgm. per cent*	*minutes*	
100	February 3	4	73.6	100.9	3	+37.0
111	March 1	3.5	89.8	125.5	10	+39.7
111	March 3	3.5	95.3	126.6	10	+32.8
111	March 15	3.6	86.4	112.2	10	+29.8
113	February 1	3.7	96.0	126.2	3	+31.4
Averages			88.2	118.3		+34.1
Sympathectomized cats						
104	February 3	5.3	98.2	90.0	3	−8.4
104	February 8	5.2	94.7	95.0		+0.4
104	February 15	5.4	84.6	83.7	2	−1.0
104	March 1	5.5	93.7	98.9	10	+5.5
104	March 8	5.4	77.1	78.3	10	+1.4
104	March 10	5.3	91.7	86.4	11	−5.9
107	March 17	2.9	77.9	78.3	10	+0.5
107	March 22	2.9	71.7	67.5	11	−6.0
Averages			86.2	84.8		−0.02

a second sample of blood was taken from the ear in the same manner as before. The analyses of these samples are set forth in table 2.

The remarkable increases in the blood-sugar percentages of normal animals as a consequence of excitement are quite in accord with the recent observations by Britton (1928). His results show an average increase of 26 per cent; in our smaller series the average increase was 34 per cent but the excitement periods as a rule were longer. The results with the sympathectomized animals were strikingly different. The variation of the glycemic percentages was much less than in the normal animals and was as

likely to be a decrease as an increase. The amount of change after the sympathetic system had been removed was so small and irregular as to be negligible.

It was a matter of interest to know what effect excitement has on blood pressure in the experimental animals. A few observations made by means of a cuff about the lower abdomen, as suggested by McGregor (1928), indicated that excitement and struggle did not have nearly the same influence in producing a high blood pressure in the sympathectomized cats as it did in the normal. In three tests on normal animals the pressure was found to vary between 190 and 210 mm. Hg. The animals were, however, greatly excited during the test. In another instance the animal was calm at first and the pressure was 135 mm.; then he became greatly excited and the pressure rose to 185 mm. In four observations on cat 336, which had been completely sympathectomized, the pressure varied between 105 and 115 mm. Hg, as the animal struggled and showed signs of pronounced excitement. Unfortunately it was impossible to make observations during a quiet state. The relatively low records after removal of the sympathetic were what might have been anticipated, in consideration of the fact that blood vessels throughout the body had been freed from any control from the central nervous system. We regret that we do not have more detailed data to report.

In connection with the evidence that in excited sympathectomized cats the blood pressure does not even remotely approach the height it reaches under the same conditions in normal animals, the failure of emotional polycythemia and relative mononucleosis after sympathectomy is of interest. Izquierdo and Cannon (1928) found an average increase of 20 per cent in red corpuscles of the blood of normal cats which were subjected to one minute of intense excitement. And Menkin (1928) observed that excitement causes a relative increase in mononuclear cells averaging 13 per cent. Both phenomena fail to occur after excision of the upper abdominal sympathetic cords and section of the splanchnic nerves or after removal of the spleen.

It is significant that absence of sympathetic nerves and failure of various important changes which ordinarily attend great excitement, had no notable influence on the vigor of the emotional reaction. As shown in figure 3, when a barking dog approached a mother cat and her kittens she behaved in all respects like a normal animal. Though erection of hair (which may be taken as a symbol of all the sympathetic effects) failed to occur, the cat, with ears back, teeth showing, and paw ready to strike, was prepared to protect both herself and her young. The psychological significance of this phenomenon has been discussed elsewhere (see Cannon, 1927).

Effect of cold on sympathectomized animals. As shown in figure 2, expos-

ure to cold after sympathectomy is not attended by erection of hair in the denervated area, i.e., the cold has no local contractile effect on the pilo-

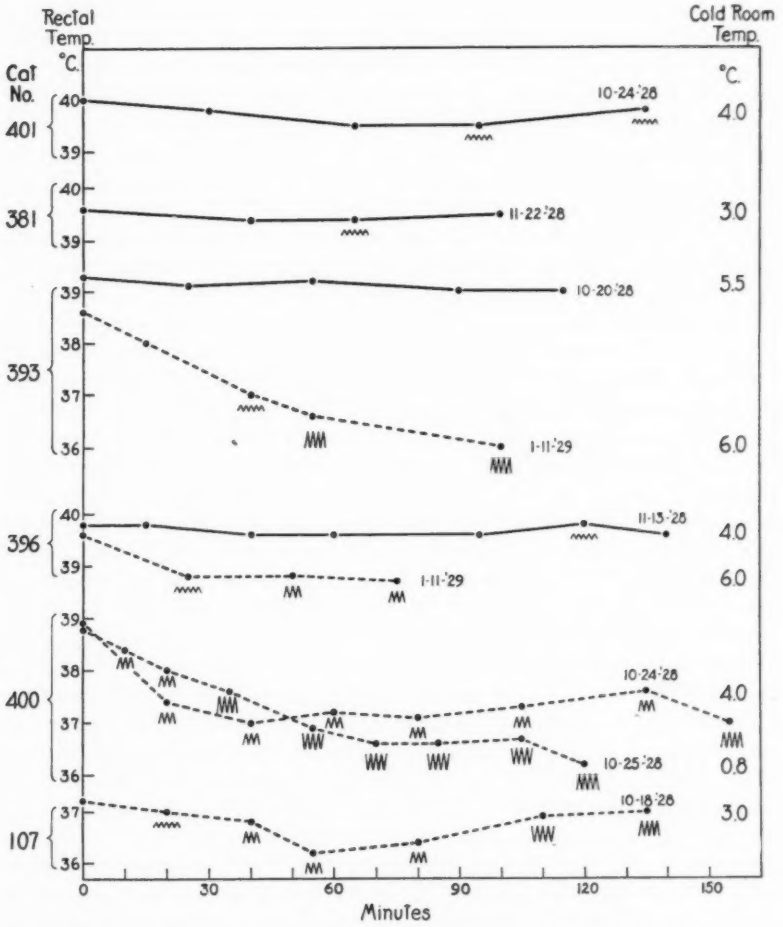


Fig. 4. Records of the rectal temperature of cats kept in a cold room. Continuous lines show the effects on normal cats; dash-lines the effects on sympathectomized cats. Shivering is represented by zig-zags under the record; the size of the zig-zags indicates roughly the intensity of the shivering reaction.

motor muscles. The smooth muscles of the blood vessels likewise are no longer under nervous government and even the earliest investigators of

vasomotor nerves noted that cold has no local contractile effect on the denervated vessels. Two important conditions for the conservation of body heat when external surroundings are cold are therefore lacking—a thicker layer of poorly conducting air cannot be enmeshed in standing hairs and the superficial circulation cannot be reduced. Necessarily the heat loss is no longer controlled. Furthermore, the organism has been deprived of the services of an increased secretion of adrenin which can serve as a primary agent to accelerate heat production when the body temperature tends to fall (see Cannon, Querido, Britton and Bright, 1927). *A priori* the sympathectomized animal is physiologically defective when confronted with the problem of maintaining its normal temperature.

The behavior of our experimental animals in cold weather was such as might be expected from the foregoing considerations. They exhibited a marked antipathy to cold air and to drafts. It has not been uncommon to see them literally spend days during the colder periods of winter crouched on top of the warm-water heater and descending from it only at feeding time.

To put these animals to a test we have taken them to a cold room, the temperature of which ranged between 0.8° and 6°C., and have watched the effect on them as compared with normal animals. Observations were made on the rectal temperature and on shivering reactions. Typical results are reported in figure 4. The solid lines trace the temperature changes of normal animals during their stay in the cold environment for about two hours; the dash-lines, the changes in sympathectomized animals under similar conditions. The observations on cats 393 and 396 were made both before and after sympathectomy. The striking differences which were noted were the much deeper depression of the rectal temperature of sympathectomized animals as compared with the normal, the rapid drop of their temperature to a low point at which it could be maintained, and the much greater frequency and prominence of shivering. Indeed, since shivering was the only resource, except exercise, that was left for the animals to protect them from further drop of temperature, the level at which temperature was held (see 396 and 400, fig. 4) probably indicates an equilibrium between the heat lost to the cold surroundings and the heat produced by the shivering process. In this relation the behavior of cat 107 is of interest. Unlike cats 393 and 396, which had been deprived of their thoracic and abdominal sympathetic chains 34 and 35 days respectively before the tests recorded in figure 4, cat 107 had lived for more than twenty months with the sympathetic practically all gone, and for more than a year with no part of the sympathetic system to rely upon (see p. 89). Very soon after cat 107 was placed in the cold room it began shivering vigorously, and when the drop from 36.8° to 36.2° occurred the muscular agitation was so pronounced that the shaking of the cage in which the animal was confined could be heard by the observer who was waiting outside the room. Doubtless as a conse-

quence of this prompt and energetic reaction to the cold the body temperature fell only one degree, and in the course of an hour it was restored to only 0.2° below the original point. It seems probable that during months of experience the organism of 107 had become accustomed to a reliance on shivering as the main protection against a temperature fall and that as a consequence shivering was a more efficient agency than in the other more recently sympathectomized animals. The fall during the tests on them, it will be noted, was much greater than in 107, although the temperature of the cold room was not so low.

From the results just reported it is clear that animals without the sympathetic system are much handicapped when conditions favor a loss of body heat. Not being able to protect themselves by conservative measures they must produce more heat in order to keep warm. Under these circumstances they must eat more food than normal animals in order to preserve their weight. It is remarkable that in spite of such demands upon them they not infrequently have become considerably heavier after being deprived of the sympathetic system. As already mentioned, cat 286, which weighed 3 kg. after the final operation, increased to 4.5 kg.—a gain of 1.1 kg. over the initial weight. And cat 104 (table 3 and fig. 5) increased from 3.59 kg. to 5.4 kg. during the course of his experience in the laboratory. Other animals did not show such noteworthy increases but they maintained their weight, as shown by the figures in table 3.

The basal metabolism after sympathectomy. The well-known effects of sympathetic impulses on carbohydrate metabolism, the possible effects of these impulses on protein metabolism (Stübel, 1920), and the involvement of the sympathetic in the metabolic adjustments which help to control body temperature, naturally raise the question whether sympathectomy would have any influence on basal metabolism. In order to obtain an answer to this question we measured the basal rate of four cats before operation and after different stages of operation A, described above.

The method used for measuring the rate in all but a few determinations was that described by Benedict and Homans (1912) and later used in this laboratory by Aub, Forman and Bright (1922). Essentially it consists of a small air-tight metal box, kept at fairly uniform temperature by a water-jacket, in which the animal is placed and through which, and through the closed analyzing system attached to it, air is made to circulate by a pump. As oxygen disappears pure oxygen is admitted through a Bohr meter. By weighing the carbon-dioxide absorbing systems before and after a period, estimating the oxygen used, and applying proper corrections, figures are obtained which permit the metabolic rate to be calculated. The last three determinations on cat 104 (table 3), the last on cat 106, and the last six on cat 107 were made by the Tissot method; the air driven through the air-tight box was collected in spirometers and samples were analyzed in a

TABLE 3

Figures for basal metabolism of cats before and after stages of sympathectomy

DATE	WEIGHT	CALORIES PER HOUR		R.Q.	TEMPERATURE	REMARKS
		Total	Per kgm.			
Cat 103						
	kg.				*°C.*	
October 17, 1925	2.84	6.49	2.23	0.821	38.6	
October 19, 1925	2.96	5.98	2.17	0.788	38.4	
October 20, 1925	2.84	6.29	2.22	0.817	38.4	
	Average		2.21			October 22, 1925, abdominal sympathetics removed
October 26, 1925	2.8	5.78	2.06	0.799	37.8	
November 4, 1925	3.02	7.00	2.32	0.887		
November 12, 1925	2.85	5.78	2.03	0.822		
November 18, 1925	3.02	7.25	2.40	0.870	38.0	
November 20, 1925	3.05	7.20	2.36	0.784	38.2	
November 24, 1925	3.40	7.97	2.34	0.885		
	Average		2.25			Cervical sympathetics removed
December 22, 1925	2.6	5.63	2.16	0.826	38.4	
January 15, 1926	2.98	5.89	1.98	0.813	38.0	
January 20, 1926	3.2	6.65	2.08	0.806	39.1	
	Average		2.07			February 6, 1926, thoracic sympathetics removed; vagi untouched
February 26, 1926	3.00	5.80	1.93	0.736	37.8	
March 1, 1926	2.99	5.98	1.98	0.785	37.8	
	Average		1.96			
Cat 104						
October 21, 1925	3.59	8.13	2.26	0.804	38.4	
October 23, 1925	3.51	6.45	1.84	0.785		
November 4, 1925	3.6	6.60	1.88	0.764		
November 11, 1925	3.49	6.27	1.80	0.775	37.9	
November 16, 1925	3.6	7.04	1.95	0.812		
	Average		1.95			November 24, 1925, both cervical sympathetics removed
November 30, 1925	3.62	6.16	1.70	0.774	37.4	
December 2, 1925	3.54	5.30	1.50	0.772	37.4	
December 3, 1925	3.78	7.17	1.89	0.829	38.2	
December 12, 1925	4.15	6.96	1.69	0.750	37.7	

TABLE 3—*Continued*

DATE	WEIGHT	CALORIES PER HOUR		R.Q.	TEMPERATURE	REMARKS
		Total	Per kgm.			
Cat 104—*Continued*						
	kg.				*°C.*	
January 13, 1926	3.9	6.23	1.60	0.733	38.8	
January 18, 1926	4.2	7.49	1.78	0.784	38.1	
January 20, 1926	4.2	6.95	1.62	0.778		
	Average		1.68			February 2, 1926, abdominal sympathetics removed; right splanchnics cut
March 2, 1926	4.5	8.31	1.85	0.774	38.6	
March 3, 1926	4.4	8.92	2.03	0.753	38.8	
March 6, 1926	4.2	7.10	1.69	0.808	38.8	
March 10, 1926	4.2	8.28	1.97	0.753	38.4	
March 12, 1926	4.05	7.79	1.92	0.779	39.0	
March 16, 1926	4.00	7.11	1.78	0.822	38.9	
	Average		1.87			March 25, 1926, both thoracic sympathetics removed
April 1, 1926	4.00	8.75	2.19	0.740	38.4	
April 5, 1926	3.88	6.96	1.79	0.852	38.0	
April 14, 1926	3.65	6.92	1.90	0.733	39.2	
April 23, 1926	3.82	6.31	1.65	0.760	38.8	
April 28, 1926	4.20	8.30	1.98	0.780	38.2	
	Average		1.90			May 4, 1926, left splanchnic and right and left semilunar ganglia removed
October 27, 1926	5.15	9.37	1.82	0.767	38.2	
December 20, 1926	5.00	7.99	1.60	0.752	38.0	
January 29, 1927	5.40	10.64	1.98	0.737	38.2	
	Average		1.80			
Cat 106						
February 8, 1926	2.6	5.44	2.09	(0.701)	38.0	
March 3, 1926	3.1	5.46	1.77	0.762	37.5	
March 4, 1926	3.2	7.01	2.19	0.767		
March 10, 1926	3.2	6.10	1.91	0.819	38.1	
March 16, 1926	3.3	6.45	1.95	0.818	37.6	
	Average		1.98			April 8, 1926, cervical sympathetics removed

TABLE 3—*Continued*

DATE	WEIGHT	CALORIES PER HOUR		R.Q.	TEMPERATURE	REMARKS
		Total	Per kgm.			
Cat 106—*Continued*						
	kg.				*°C.*	
April 13, 1926	2.4	4.16	1.73	(0.674)	38.2	In good condition
April 15, 1926	2.4	3.98	1.66	(0.704)	38.2	
April 27, 1926	2.5	5.32	2.13	0.798		
April 29, 1926	2.48	4.39	1.77	0.822	38.5	
	Average		1.82			May 4, 1926, abdominal sympathetics, *except splanchnics*, removed
May 8, 1926	2.25	4.17	1.85	0.773	37.6	
May 8, 1926, p.m.	2.25	4.93	2.19	0.759	38.4	
May 19, 1926	2.25	4.39	1.95	(0.635)		
May 20, 1926	2.20	3.86	1.75	(0.610)	37.0	
June 10, 1926	2.30	4.29	1.87	0.754	37.6	
June 11, 1926	2.40	5.11	2.13	0.814		
	Average		1.96			June 18, 1926, thoracic sympathetics removed
June 29, 1926	2.03	3.71	1.83	0.772	38.0	
October 28, 1926	2.9	5.66	1.91	0.937	38.1	
	Average		1.87			
Cat 107						
March 4, 1926	2.8	5.03	1.80	0.861	38.6	
March 5, 1926	2.7	4.95	1.83	0.846	38.2	
March 9, 1926	2.88	5.77	2.00	0.853		
March 17, 1926	3.00	6.04	2.01	0.833	38.4	
March 19, 1926	2.90	5.10	1.76	0.796		
March 22, 1926	3.0	5.59	1.86	0.750	38.0	
	Average		1.88			April 8, 1926, abdominal sympathetics removed except major splanchnics
April 13, 1926	2.83	5.98	2.11	0.723	39.4	
April 23, 1926	3.25	6.10	1.88	0.734	38.4	
May 4, 1926	3.03	5.38	1.78	0.818	38.2	
May 6, 1926	2.95	4.95	1.68	0.782	38.4	
	Average		1.86			May 11, 1926, cervical sympathetics removed

TABLE 3—*Concluded*

DATE	WEIGHT	CALORIES PER HOUR		R. Q.	TEMPERATURE	REMARKS
		Total	Per kgm.			
Cat 107—*Concluded*						
	kg.				°C.	February 4, 1927, both thoracic sympathetics removed; weight cat, 3.35 kgm. March 1, 1927, both splanchnics and semilunar ganglia removed
March 14, 1927	2.85	5.03	1.61	(0.679)	37.9	
March 16, 1927	2.9	4.68	1.61	0.809		
March 23, 1927	3.0	5.81	1.95	0.771	38.6	
April 5, 1927	2.82	4.58	1.63	0.898		
June 14, 1927	3.0	4.89	1.63	0.783		
		Average	1.69			

Haldane gas analyzer. The two methods were frequently checked by the burning of alcohol in the box. A delicate recording apparatus which supported the box registered graphically any motions of the animal. Whenever in any period the animal was even slightly restless the data for that period were discarded.

The remarkable feature of the results recorded in table 3 is the relatively slight change in metabolism produced by sympathectomy, at any stage or when complete. The average normal basal rate, before any operation was performed, was higher than any later average rates in all animals except 103; in that animal the average after abdominal sympathectomy was practically the same as in the normal stage. It is interesting to note that removal of the cervical sympathetic chains appeared to have a larger effect than removal of the abdominal chains; in cats 104 and 106, the primary excision of the cervical chains dropped the average metabolic rate 14 and 8 per cent respectively, whereas in cats 103 and 107 the primary excision of the abdominal chains (except the splanchnics) had no marked effect. When the cervical chains were taken from 103 it is noteworthy that the rate again went down. Apart from the 14 per cent drop after cervical sympathectomy in cat 104, however, the greatest drop from the normal figure was only 11 per cent (2.21 to 1.96 cals. per kg. per hr. in 103). In other instances the average figure for the final stage showed a fall of 8 per cent (in 104), 6 per cent (in 106), and 10 per cent (in 107). There was a definite, though slight, decrease in the basal metabolic rate.

The decrease in the metabolic rate as more of the sympathetic system was removed poses the question whether mere existence under laboratory conditions for a long period would not have a depressant effect by itself. Lusk (1915) has called attention to the influence of confinement in a cage in bringing about a noteworthy reduction in the basal metabolism of the dog—a condition which is recovered from by exercise. Our animals were not kept in cages, however, but had free range of a room of considerable size. Furthermore, a normal cat, kept as a control under the same conditions, that had a basal metabolic rate of 1.83 calories per kilo per hour on January 21, 1926, and 1.63 on March 11, had a rate of 1.96 on October 26 and 1.88 on November 30 of the same year. After March 11 there was no note-

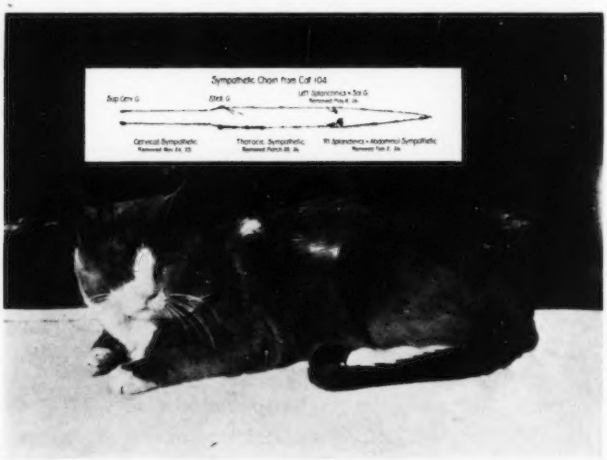


Fig. 5. Photograph of cat 104 taken February 12, 1927, nine months after removal of the semilunar ganglia and the last portion of the sympathetic chains. The parts removed were mounted on the card above the cat.

worthy change of weight—it varied from 3.8 to 3.85 kg. in eight months. Certainly in that animal life in the laboratory had no depressant influence on basal metabolism. Again, Aub, Forman and Bright (1922) have reported a laboratory animal which they studied for seven months and in in which "the metabolism per kilo remained essentially constant throughout." Rapport's record (1924) of repeated tests on a dog during fifteen months of existence under laboratory conditions likewise emphasizes the constancy of the basal metabolic rate. Undoubtedly our best case is that of cat 104 (see fig. 5). As shown in table 3, tests were made on that animal from October 21, 1925, to January 29, 1927. There was, as already noted, a drop of 14 per cent in the basal metabolism after cervical sympathectomy

but that was recovered from and each successive step thereafter in the eradication of the sympathetic system was followed by an average basal rate less than 8 per cent variant from the original normal average.

In brief we may state that although cervical sympathectomy appears to have a temporary depressant effect on the basal metabolic rate which may be noteworthy, removal of other parts, until the entire system has been excised, results in a diminution of the metabolic rate so small as to be probably not significant.

Some general observations. A number of incidental facts noted in our animals may be of interest.

We have not seen any typical change in the skin after either unilateral or bilateral sympathectomy. Mange has occurred, but not more frequently than in normal animals kept in the laboratory. There has been no edema, ulceration or other disturbance of the body surface at any point. Saalfeld (1901) reported that hair grows more rapidly after sympathectomy. We have not measured the rate of hair growth, but we can testify that there is no obviously greater length of the hair on the denervated side at any stage in developing kittens, and no notably longer hair in animals completely deprived of their sympathetic system.

The snout feels warm in healthy sympathectomized animals, in contrast to the usual coolness of that surface. A rather more abundant flow of mucus or watery fluid from the nostrils than in normal animals is commonly observed. Our experimental animals had respiratory infections ("snuffles") as did other animals in the laboratory, but on being confined each to a small cage and kept warm by radiant heat from a hot electric coil they usually recovered.

Immediately after operative removal of the stellate ganglia or the cervical sympathetic chains the nictitating membranes were prominently relaxed over the eyeball. This condition persisted for many days, but it gradually disappeared. The phenomenon was noted by Langley and also by Dresbach andWaddell (1926) after excision of the stellate ganglia. The disappearance of the membranes need not imply a reconnection of the inferior cervical ganglion with the central nervous system, as Dresbach and Waddell stated, for that was an impossible condition in our experiments. In all probability the phenomenon was due to the development of an intrinsic tone in the smooth muscles of the third eyelid, analogous to what may occur in smooth muscle elsewhere.

As shown in figure 3, a cat deprived of the sympathetic system exhibits, under appropriate circumstances, all the typical external signs of aggressive feeling (except erection of hairs). Cats have very various dispositions, some are ugly and savage, some friendly and affectionate, some restless, some quiet and serene, some playful, some indolent. Removal of the sympathetic nervous system, in our experience, makes no change in the natural disposition of the animal.

Discussion. Shortly after the preliminary account of the work here reported in detail was published, the question was raised as to whether complete sympathectomy could be achieved (Jelliffe, 1927). Although this question has already been considered (see p. 86) it is perhaps necessary to reiterate that we are using the term "sympathetic" not as equivalent to "vegetative" but as a brief designation of the thoraco-lumbar division of the autonomic system. Since the bilateral chains of ganglia, from the superior cervical to the pelvic, and in addition the semilunar ganglia, contain the cell bodies of all sympathetic neurones distributed to the iris, the nictitating membrane, the digestive glands, the heart, the wall of the alimentary tract, the urogenital system, the blood vessels of all parts of the body, and the hairs and sweat glands, the sympathetic fibres to these structures must have degenerated. In the absence of the sympathetic ganglia, as Langley and Anderson (1904) have shown, the preganglionic fibres—parts of neurones with cell bodies inside the central nervous system —cannot make effective connection with smooth muscles and glands. No restoration of sympathetic nervous control, therefore, is possible. Consequently smooth muscle and glands throughout the body are permanently deprived of their sympathetic nerve supply and of stimuli from the brain and spinal cord.

Recent medical literature has many references to hypothetical "vagotonic" and "sympathicotonic" states, and to "autonomic imbalance." The concept underlying the invention of these terms is that the sympathetic and the cranial divisions of the autonomic system are acting constantly in opposition to each other and that the resultant of the conflict is an equilibrium between the two. There is evidence of opposition in some organs, but not everywhere. For example, there is no evidence that sympathetic impulses are acting tonically to inhibit the muscular functions of the stomach and intestines—splanchnic section does not accelerate the passage of material through the canal (see Cannon, 1906). There is no evidence that these impulses act tonically to check the operations of the digestive glands, or to maintain a continuous slight contraction of the muscles of the hairs. It could hardly be expected that exclusion of sympathetic influences could bring about a condition of "autonomic imbalance" in these regions. The chances are more favorable where there is some indication of sympathetic tone. For a time after sympathectomy the pupils are constricted, the nictitating membrane extends outward from its corner of the eye, the heart rate is slower, and probably the arterial blood pressure is somewhat reduced. These conditions might be seized upon as evidence of the "vagotonia" which must result when sympathetic tone is banished. But the heart rate is slow after exclusion of the accelerators if the vagi also are cut. Moreover the changes which follow excision of the sympathetic ganglia are not permanent as they might reasonably be expected to be if antagonistic

impulses were thus left unopposed. The nictitating membrane is gradually retracted, the pupil becomes less constricted and the denervated blood vessels probably regain to some degree a local tonic state (Langley, 1900). In short, pathological symptoms are remarkable for their absence. Thus when conditions are actually produced in the body that would most effectively induce "vagotonia" and "autonomic imbalance," the phenomena attributed to that state are local, slight and temporary.

Recent discussion of possible sympathetic control of the tonus of skeletal muscle (Langelaan, 1915; see also Forbes, Cannon, O'Connor and Miller, 1926) and of its service in resisting fatigue (Orbeli, 1924) raises questions as to the influence of sympathectomy on muscular function and performance. We have not seen any sign of any difference of use of skeletal muscles by completely sympathectomized cats as compared with normal animals, or by use of the muscles on one side as compared with the other after a unilateral operation. In the latter case the knee jerks on the two sides are equal. We have not tested our cats for duration of muscular performance, for they do not run well in a treadmill, but unilateral sympathectomy of the lower abdomen in the dog neither reduces the total performance nor brings out any difference of action of the two limbs (Campos, Cannon, Lundin and Walker, 1929).

In surveying the results reported and discussed in the foregoing pages we have been repeatedly impressed by the relatively slight effects resulting from removal of the sympathetic system. This system belongs to what has been called the vital nervous system ("Lebensnervensystem," Müller, 1924), governing the proper course of functions necessary for continued existence. And yet we find no indication that animals after they have been deprived of it suffer more frequently from disease or succumb more readily when infected than do normal animals. In the quiet conditions of the laboratory they are indistinguishable from their fellows in appearance and behavior. At first thought it might seem that the sympathetic system is of minor importance for proper bodily functioning. We believe that such an inference is erroneous. That system is brought into operation prominently whenever hostile or harmful influences in the environment become dangerous for the organism—when circumstances induce fear, rage, pain, depressed temperature or need for oxygen. In such critical conditions the sympathico-adrenal system is of primary service for adjusting the internal environment to the special and urgent needs of the body; it is in its *emergency* function that the system has its value for the individual (see Cannon, 1928). The animals in the laboratory do not have to meet the exigencies of normal existence in the outer world. If they should be set free and a critical situation requiring vigorous effort should arise, there would be no secretion of adrenin, no rise in blood sugar, no polycythemia, no marked splanchnic vasoconstriction with consequent rise of blood pressure and

faster blood flow, no great acceleration of the heart, no shifting of the circulation to benefit the contracting muscles. It is extremely doubtful if a sympathectomized animal could protect itself with the efficacy of a normal animal in which these beneficial changes occur. In rapidly adjusting the internal organization for efficiency in meeting sudden external demands on the body the sympathetic system, we believe, has its significance.

SUMMARY

Methods are described for removing both sympathetic chains from the superior cervical to the pelvic ganglia in parts (see figs. 3 and 5) or from the stellate to the pelvic ganglia unbroken (see fig. 1). The completeness of the elimination of sympathetic control resulting from the operation is shown in figure 2.

Bilaterally sympathectomized animals have lived in the laboratory in good health, performing normally all the routine functions, for many months (see fig. 5). The claims that the superior cervical sympathetic ganglia, or the mesenteric nerves are essential for life are thus disproved. Additional removal of one adrenal and demedullation of the other demonstrates that the chromophil tissue is not of vital importance.

Unilateral sympathectomy of young kittens has not resulted, as they have grown to adult size, in any demonstrable differences in bilaterally symmetrical organs (see table 1).

Sympathectomy does not prevent the female from performing the functions of reproduction and lactation (see fig. 3).

After bilateral sympathectomy emotional excitement causes no erection of hairs (see fig. 3), no consistent increase of blood sugar (see table 2), no polycythemia, no relative increase of mononuclear cells, and, as shown by a few observations, no marked rise of arterial blood pressure.

Sympathectomized animals are very sensitive to cold; having lost the means of conserving heat they seek warm places, and when placed in a frigid environment they lose heat more rapidly than normal animals (see fig. 4).

The basal metabolism usually falls somewhat after sympathectomy, especially after the cervical portion is excised, but as a rule removal of the sympathetic chains does not reduce the basal metabolic rate more than 10 per cent (see table 3).

The relation of these experiments to "vagotonia" and "autonomic imbalance" is discussed.

The slight effect resulting from sympathectomy raises the question as to the function of the sympathetic. This question is considered with regard to the natural conditions which excite the sympathico-adrenal system, and the conclusion is drawn that this system, dispensable in the protected conditions of the laboratory, finds its great service at times of critical emer-

gencies when it adjusts the internal organs of the body for use of the mechanisms responding to external exigencies.

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STUDIES ON NERVE METABOLISM

V. Phosphates

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That carbohydrates enter into the resting and active metabolism of isolated peripheral nerve has been demonstrated in previous papers of this series (see Holmes, Holmes and Gerard, 1929). It has also appeared from a theoretical consideration of the effects of asphyxia and of continued stimulation, as well as from the formation of ammonia (Gerard and Meyerhof, 1927), that other intermediate chemical mechanisms were involved in the normal behavior of nerve (Gerard, 1927b). A natural next step was the investigation of the nerve phosphates, because of their long recognized importance in carbohydrate metabolism and because of the recent evidence of their separate importance in relation to muscle.

Methods. *a. Determination of phosphate.* The method of Fiske and Subbarow (1925) as applied by Lohmann and Jendrassik (1926) has been used almost entirely for the actual colorimetric estimation of phosphate. A small Bio-Klett colorimeter served for most of the work. The preparation of the various fractions was accomplished in the manner described by Lohmann (1928). The nerves were thoroughly ground with iced trichloracetic acid (usually 12 cc.), allowed to stand 10 to 15 minutes, filtered, and three aliquot portions of the filtrate taken. In several experiments the nerves were pulverized while frozen with liquid air before adding the trichloracetic acid, and since the results were unchanged we may consider the acid extracts complete. One fraction (2 cc.) was ashed with sulphuric and nitric acids to give the *total phosphate*, determined colorimetrically. This refers, of course, to total soluble phosphates, the phosphatids being excluded except as they might break down to give soluble phosphate fractions.

The second fraction (3 cc.) was directly analyzed for phosphate using the strongly acid reagent and warming for 5 minutes at 38°C. This insured the complete breakdown of any creatine-phosphate (phosphagen) present, so that the inorganic and creatine-phosphate were determined together. This fraction we have called the *free phosphate.*

[1] Aided by a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

The true *inorganic phosphate* was determined in the third fraction (5 cc.) either 1) directly after precipitation as $MgNH_4PO_4$ by strong ammonia and Mg citrate[2] and washing away the combined phosphates remaining in solution, or 2) by following the curve of breakdown of phosphagen (course of color development) and extrapolating to zero time. For the latter procedure both the Fiske and Subbarow (1927) and the Briggs methods (as followed by Eggleton and Eggleton, 1927) were used. Both inorganic and creatine-phosphate were determined on the same fraction in this case but despite the larger quantities thus available for each, the alkaline precipitation seemed more satisfactory in our hands.

From the three values for a sample its content of each form of phosphate is determined. The inorganic phosphate is directly measured, the acid-labile phosphate is given by the difference between "free" and inorganic, and the acid-stable phosphate by the difference between total and "free." We have no basis for identifying the two combined phosphate fractions beyond calling them acid-labile and acid-stable, but it seems a reasonable assumption that these correspond to the similarly acting compounds in muscle. We may then speak of them respectively as creatin-phosphate and hexose-phosphates.[3]

The phosphate content of nerve is considerably less than of muscle from the same species, and the available amount of nerve is very much less than of muscle. We have been forced, therefore, to determine relatively small

[2] In addition to the precautions for securing complete precipitation mentioned by Lohmann, we have placed a small tuft of glass wool in the precipitation tubes to aid crystallization.

[3] Lohmann (1928a) has recently shown that nearly 50 per cent of the acid-stable fraction or 15 per cent of the total soluble phosphate of frog muscle is pyrophosphate, and 6 per cent of the total in rabbit's brain (Lohmann, 1928b). We have found pyrophosphate (by Lohmann's test) present in frog nerve in a duplicate experiment on 12 frogs as follows: free P 19 mgm. per cent and 19.5 mgm. per cent, free and pyrophosphate P 22.5 mgm. per cent and 23 mgm. per cent, total P 27 mgm. per cent and 28 mgm. per cent. One-third of the acid-stable fraction (29 per cent) is pyrophosphate (12 per cent of total). Only 15 per cent of the pyrophosphate in muscle is broken down by prolonged asphyxia and less than half by excessive stimulation (Lohman, 1928c).

We cannot say what portion of the remaining 5 to 6 mgm. per cent of acid-stable phosphate may be in the form of nucleotides. (See Embden, 1928, Parnas, 1928a.) Pohle (1928) reports the presence of adenylic acid in brain, but Parnas (1928b) finds in nerve no traumatic NH_3 production, which appears to be a rough index of the presence of adenylic acid. Holmes, Holmes and Gerard, 1929, obtained tests for pentose in nerve, and Wen and Gerard (unpublished) have evidence of small traumatic ammonia production, but if all from adenylic acid this would represent hardly more than 1 mgm. per cent of phorphorus in this form. In any event, since there is no evidence in muscle that the nucleotides are split to nucleosides and inorganic phosphate under the various conditions of experimentation, we may for the present assume this factor constant.

amounts of phosphate. In the final test solution there was usually present 0.01 to 0.03 mgm. of P in 25 cc. (sometimes 15 cc.), which yields only a pale blue color. We have been able by repeated tests, however, to convince ourselves of the dependability of our colorimetric readings.

That no phosphate is systematically lost in the various procedures has also been demonstrated by repeated control tests with known solutions and with known amounts of phosphate added to the nerves at the start. Table 1 shows some of the controls performed and indicates the degree of accuracy obtained. This is naturally lower than would be the case for larger quantities but the error is well below the range of observed variations. The consistency of results on equivalent sets of nerves further attests the dependability of these procedures even for very small quantities.

b. Preparation of nerves. Sciatic nerves of rabbits, bull frogs (Rana catesbiana) and ordinary green frogs (R. Temporia and R. Pipiens) were used. The frogs were handled as previously described (Gerard, 1927a), but the nerves were placed as soon as dissected in a dry mortar on ice. When the required number of nerves was accumulated (usually six nerves in each batch, taking 20 to 30 minutes to prepare) they were weighed on a Hartman-Braun torsion balance and returned to the mortar for immediate grinding or kept under varying experimental conditions before grinding. Controls showed that the period of cooling and exposure did not affect the condition of the phosphates. A 5 per cent loss in weight occurs in this time, which has not been corrected for in the figures given.

Rabbits were killed by a sharp blow on the head, the hind legs skinned and the sciatics and their larger branches (the peroneal nerve down to the ankle) dissected free. Especial care was taken to avoid stretch or other injury to the nerves but this prolonged the dissection time for each nerve to 10 or 15 minutes. At 37°C. with the circulation stopped through the nerve, considerable change from the true resting state may have occurred in this time. In the preparation of all nerves the attempt was made to minimize mechanical trauma, contamination with blood, contact with injured muscle, drying, and time between killing the animal and exposing the nerve to the oxygen of the air.

The nerves kept for varying times in O_2 or N_2 were spread (to insure adequate diffusion) on the sides of glass tubes closed below by a stopcock and above by a ground glass stopper. Platinum electrodes were sealed in the side. A small amount of Ringer solution was kept in the bottom and the gas bubbled through from below, to prevent drying of the nerves. Oxygen was used directly from the cylinder, nitrogen after passage over glowing copper shavings and washing in a weak alkaline hydrosulphite solution. Nitrogen was bubbled through the nerve chamber rapidly at first, then slowly for 10 minutes, the chamber being closed with a slight positive pressure.

By the procedure outlined, the nerves were kept moist without ever being soaked or washed with Ringer solution, so almost no loss of phosphates by diffusion could occur. Sometimes the nerves were directly moistened with a phosphate free Ringer with no different results. Tests failed to show any phosphate on the walls of containers where nerves had been resting.

For stimulation, a Harvard inductorium was used with 2 volts in the primary, a special hammer giving 300 shocks per second (make and break), and the secondary coil at 12 cm. set horizontal. Tests by means of action

TABLE 1

Controls

ADDED P	METHOD	STANDARD	COLORIMETER READING	ERROR
mgm.				*per cent*
0.008	Fiske and Subbarow direct	0.016 at 15 mm.	29.2	+2.7
0.016	Fiske and Subbarow direct	0.016 at 15 mm.	15.0	0.0
0.032	Fiske and Subbarow direct	0.016 at 15 mm.	7.7	−3.8
0.040	Fiske and Subbarow direct	0.04 at 15 mm.	15.1	−0.7
0.040	Fiske and Subbarow direct	0.04 at 30 mm.	29.8	+0.7
0.008	5 cc. of 5 per cent trichloracetic acid present	0.008 at 15 mm.	15.1	−0.7
0.016	5 cc. of 5 per cent trichloracetic acid present	0.016 at 15 mm.	14.8	+1.3
0.024	5 cc. of 5 per cent trichloracetic acid, all "ashed" with 1 cc. 2N H_2SO_4 and 1 drop fuming nitric	0.024 at 15 mm.	14.6	+2.7
0.016	Precipitated as $MgNH_4PO_4$ and separated	0.016 at 20 mm.	18.2	+9.0
0.024	Precipitated as $MgNH_4PO_4$ and separated	0.024 at 20 mm.	20.3	−1.5

		mgm. P found
Trichloracetic extract, direct P determination	6 nerves	0.009
	6 nerves + 0.01 mgm. P	0.022

currents showed this strength of stimulus to be definitely but not greatly supramaximal. The condition of the nerves, as indicated by action currents, remained good during the experiments. For stimulation the nerves were arranged on the platinum electrodes fused in the chamber so that the central ends were stimulated, thus activating all the fibres.

The nerve chambers when filled were immersed in water at a desired temperature. It was difficult to maintain temperatures of 15°C. constant during hot weather, and variations of 2° often occurred during a long experiment. The use of a large vacuum flask as container was quite satisfactory.

RESULTS. *a. The phosphate content and distribution of fresh nerve.* There is some variation from individual to individual, more from season to season and most from one species to another in the total soluble phosphate in fresh nerve, and to a less extent, in its fractionation. Equivalent batches of nerves from the same frogs show good agreement. Table 2 reproduces a few individual experiments, and tables 6 and 7 include the gross averages.

Our values for fresh nerve probably do not represent the resting state during life, for a period of partial asphyxia and some excitation are unavoidable in the course of killing the animal and dissecting the nerves.

TABLE 2

Fresh nerve

DATE	NERVES	P MILLIGRAMS PER CENT				
		Free	Total	Inorganic	Labile	Stable
6/14/26	4 Temporaria	19	29			10
6/21/26	4 Temporaria	17	22	10	7	5
3/24/28	6 Pipiens	17	30			13
3/28	6 Pipiens	22	30			8
4/4	6 Pipiens	12	17			5
4/18	*6 Pipiens	21	29			8
	*6 Pipiens	21	29			8
4/27	6 Pipiens	25	34			9
7/24	6 Pipiens	28	37	19	9	9
7/26	6 Pipiens	34	39	16	18	5
7/31	1 Catesbiana	18	29	9	9	11
4/21	1 rabbit	14	27			13
7/27	1 rabbit	15	28	9	6	13

* Brace indicates sets of nerves from the same frogs in all tables.

Evidence in this direction is available from the changes in phosphate fractionation during rest in oxygen.

b. Rest in oxygen. Nerves kept moist in an oxygen atmosphere, as compared to their companion nerves killed at once, showed slight but fairly regular changes in their phosphates. The total amount of soluble phosphate was increased 5 to 10 per cent and the amount in combined form increased at the expense of the inorganic fraction. Table 3 contains typical results: tables 6 and 7 the averages.

c. Rest in nitrogen. In nerves kept under anaerobic conditions there is regularly a marked breakdown of combined phosphate, both acid labile and acid stable, to the inorganic form, as compared to the fresh nerve. There

is about a 15 per cent increase in the total soluble phosphate. See tables 3, 4, and 7.

d. Rest in nitrogen followed by oxygen. Equivalent nerves were kept at rest in nitrogen for equal times, one set then analysed, the other kept for a further time in oxygen before analysis. The second set regularly showed a

TABLE 3

Rest in oxygen or nitrogen

DATE	NERVE	TEMPERATURE	GAS	DURATION	P (MILLIGRAMS PER CENT)				
					Free	Total	Inorganic	Labile	Stable
6/14/26	4 Temporaria	15°	O_2	At once	17	22			5
				15 hours	17	23			6
			N_2	At once	19	29			10
				15 hours	27	31			4
4/18/28	6 Pipiens	15°	O_2	At once	21	29			8
				20 hours	18	31	•		13
			N_2	At once	21	29			8
				20 hours	27	31			4
7/16/28	6 Pipiens	15°	O_2	At once*	25	39	24	1	14
				2 hours	29	43	22	7	14
			N_2	At once*	27	42	27	0	15
				2 hours	36	43	29	7	7
			O_2	5 hours	32	43	22	10	11
			N_2	5 hours	39	42	37	2	3
			O_2	8 hours	29	44	25	4	15
			N_2	8 hours	38	42	39	0	4
7/27/28	1 rabbit	26°	O_2	At once	15	28	10	5	13
				1.5 hours	20	30	18	2	10
			O_2	3 hours	19	27	10	9	8
			N_2	3 hours	24	31	24	0	7

* Atypical for fresh nerves, considerable stimulation in removal.

"recovery" in oxygen the state of the phosphate being that of a nerve rested in oxygen from the start. The asphyxial breakdown was completely reversed. See tables 4, 6 and 7.

e. Stimulation in oxygen. Nerves stimulated in an oxygen atmosphere regularly showed an increase in inorganic phosphate at the expense of

the combined, when compared with equivalent nerves examined fresh or after an equal period at rest in oxygen. This breakdown of combined phosphate was less than the maximum obtained in asphyxia. See tables 5, 6 and 7.

TABLE 4

Recovery in oxygen after nitrogen

DATE	NERVE	TEMPERATURE	HOURS IN N_2	HOURS IN O_2	P (MILLIGRAMS PER CENT)				
					Free	Total	Inorganic	Labile	Stable
7/26	6 Pipiens	15°	2½		32(?)	31	22	10(?)	0
			2½	6	24	28	18	6	4
			2		24	39	16	8	15
			2	6	21	34	9	12	13
7/31	1 Catesbiana	26°	1½		20	26	12	8	6
			1½	3	17	26	7	10	9
			Fresh		18	29	9	9	11

TABLE 5

Effect of stimulation

DATE	NERVE	TEMPERATURE	HOURS	IN AIR DURING	P (MILLIGRAMS PER CENT)				
					Free	Total	Inorganic	Labile	Stable
5/10	6 Pipiens	20°	¾	Rest	25	34			9
			¾	Stimulation	28	35			7
5/16	6 Pipiens	20°	6	Rest	16	25			9
			6	Stimulation	19	26			7
5/31	6 Pipiens	20°	3	Rest	23		11	12	
			3	Stimulation	24		22	2	
6/4	6 Pipiens	19°	2	Rest	17		11	6	
			2	Stimulation	21		15	6	
			1	Rest	21		9	12	
			1	Stimulation	21		15	6	

f. Time course of the changes. A number of experiments to determine the time course of these changes at 15° to 25° have not yielded entirely satisfactory data. The variation from one set of nerves to another, and the error of individual determinations is too great to permit an accurate

statement. Figure 1 illustrates the type of results obtained for rest in oxygen and in nitrogen. Nearly all the change occurs within the first five hours or less. The averages in table 6 similarly show but little change

TABLE 6

Summary

NERVE	AVERAGE HOURS REDUCED TO 15° FOR D = 2			P (MILLIGRAMS PER CENT)					PER CENT OF P AS		
	In N_2	In O_2	Stimulation O_2 or air	Free	Total	Inorganic	Labile	Stable	Inorganic	Labile	Stable
Spring		Fresh		18 (3)*	24 (3)	10 (1)	8	6	42	33	25
Tempo-		18		16 (2)	23 (2)	7 (1)	9	7	30.5	39	30.5
raria	22			27 (7)	29 (6)	19 (3)	8	2	65.5	27.5	7
Winter		Fresh		19 (15)	27 (15)			8			30
and Spring		5		22 (16)	31 (10)	12 (6)	10	9	39	32	29
Pipiens	22			26 (6)	31 (5)			5			16
			3½	24 (12)	32 (5)	18 (7)	6	8	56	19	25
Summer		Fresh		28.5 (4)	39 (5)	20 (5)	8.5	10.5	51	22	27
Pipiens		6		30 (6)	41.5(6)	23 (6)	7	11.5	55	17	28
	2			33 (8)	43.5(8)	28 (8)	5	8.5	67.5	12	20.5
	8			37 (5)	44 (5)	32 (5)	5	7	73	11	16
	2	7		30 (6)	43 (5)	20 (6)	10	13	47	23	30
			1½	38 (2)	40 (1)	25 (2)	13	2	62.5	32.5	5
Rabbit		Fresh		14 (5)	25 (5)	9.5(1)	4.5	11	38	18	44
		5		19 (3)	28 (3)	10 (1)	9	9	36	32	32
	5			25 (3)	31 (3)	21 (1)	4	6	68	13	19

* The figure in parenthesis gives the number of experiments averaged; each experiment using nerves from six frogs or one rabbit.

TABLE 7

Weighted averages for frog nerves

CONDITION OF NERVE	P AS		
	Inorganic	Labile	Stable
	per cent	*per cent*	*per cent*
Fresh	48	24	28
In O_2 8 hours	45	26	29
In N_2 12 hours	70	15	15
In N_2 2 hours, in O_2 7 hours	47	23	30
Stimulated 2 hours	58	21	21

in the amount of combined phosphate between 2 and 8 hours in nitrogen. The changes accompanying activity also appear to be progressive for two or more hours at the temperature used.

Discussion. An extensive litrature has accumulated on the relation of phosphates to muscle activity, especially as associated with its carbohydrate metabolism. This work (Embden and Jost, 1928; Meyerhof and Lohmann, 1928; the Eggletons, 1927b; Sacks and Davenport, 1928; Nachmansohn, 1928; Fiske and Subbarow, 1927, and others) has shown that inorganic phosphate in muscle increases at the expense of hexose-phosphate (though the total acid-stable fraction may increase) and of creatin-phosphate under conditions of asphyxia and activity, and that a more or less complete reëstablishment of the initial state accompanies recovery in oxygen or by rest.

In previous papers of this series it has been demonstrated that nerve possesses a resting metabolism similar to that of muscle, and depends on

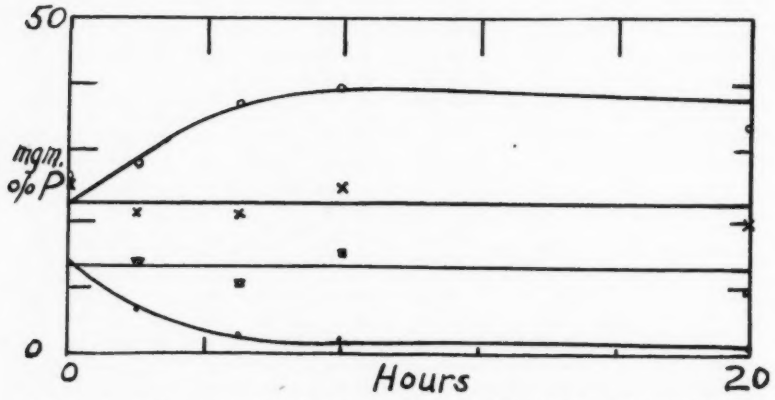

Fig. 1. ○— ○ Inorganic P in N_2; ×—× Inorganic P in O_2; ●—● Acid stable P in N_2; ■— ■ Acid-stable P in O_2.

Expt. 7/16/28. R. Pipiens. 15°C. Each point represents a determination on six nerves (see table 3)

familiar metabolic processes for its energy during activity. Carbohydrate oxidation is one of these, possibly the main one. Further, a consideration of the phenomenon of equilibration in nerve suggested the existence of intermediate reactions involving at least one other substance besides the main energy yielding one. It was a natural first guess to look for phosphate changes in nerve along the lines well established for muscle. The only previous study of nerve phosphates that we have found is one by Hecker (1923) on the total phosphates after ashing.

a. Relation to muscle. The results have been roughly what might be anticipated by analogy, asphyxia or activity leading to breakdown of combined phosphates, rest in oxygen to their resynthesis. The phosphate resynthesis following asphyxia certainly does not depend on the reformation of sugar from lactic acid or even oxidation of the lactic acid.

The amount of free sugar is steadily decreasing in nerve during rest in oxygen and is still less after a period in nitrogen (Holmes, Holmes and Gerard, 1929), whereas the creatin content presumably is not lessened. It is interesting to note that the ratio of phosphorus in stable form to that in labile form does not decrease as the ratio of sugar to creatin does, but if anything increases. The recombination of inorganic phosphate with one or other of these molecules is thus not determined by the amounts present. In muscle, Lehnartz (1928) has found it possible to profoundly alter these ratios by the action of adenylic acid.

It is doubtful, however, if these changes are as significant in relation to nerve as they are to muscle. The total soluble phosphates in frog's nerve are in much smaller concentration than in frog's muscle (25 to 40 mgm. per cent of P for nerve against 110 for muscle-temporaria) although the total P content of nerve is much higher, due to the phospholipins present. Actually the soluble phosphorus of nerve is only about 6 per cent of the total amount of this element present, as determined by ashing the entire nerve. For example, 18 temporaria nerves (0.8 gm.) contained 4.1 mgm. P per gram[4] of fresh nerve and 0.24 mgm. total soluble P per gram. Since, however, myelin occupies one-half of the bulk of nerve fibres (Donaldson and Hoke, 1905), if the soluble phosphates are only in the aqueous protoplasm their concentration in nerve would be close to that in muscle.

b. Phosphatides. The soluble phosphate of nerve tends to increase during activity and asphyxia, and possibly even at rest in oxygen. This may come from the breakdown of phospholipins, phosphoproteins or nucleins. We have performed no experiments to determine its source but because of the relative amounts present, the former would seem the most probable. The total soluble phosphate is determined by ashing the trichloracetic acid extract of the nerve, and any phospholipins carried in suspension in this filtrate would contribute phosphorus to the final value and lead to falsely high results. A change in state of the nerve colloids in asphyxia, etc., might conceivably cause such imperfect separation and so give an apparent increase in the soluble phosphorus. This cannot be absolutely excluded, but since most of our filtrates were perfectly clear and occasional cloudy ones did not yield high phosphate values, we do not believe this error occurred. The increase in soluble phosphorus of 4 mgm. per cent would represent a breakdown of over 100 mgm. per cent of phospholipins. If the phospholipins do enter actively into nerve metabolism, some interesting possibilities are opened up.

c. Equilibration. The falling off to a low constant value of the response of the frog's sciatic on continued stimulation is practically complete in the

[4] This is twice the amount found by Hecker.

first 15 minutes. This was interpreted (Gerard, 1927b) as the time necessary for equilibrium to be established between breakdown and reformation of some substance necessary to activity. Thus, in the equations:

$$CA \xrightarrow{(O_2?)} A + X \text{ (during conduction)}$$
$$C + A + E \xrightarrow{(O_2?)} CA \text{ (during the refractory period)}$$
$$X + O_2 \rightarrow CO_2 + E \text{ (during the prolonged recovery)}$$

if all CA present is broken down with each impulse carried and is reformed during the refractory period at a rate depending on the amount of E available, this latter factor will control the distribution of A between the free and combined forms. But the final reaction is very slow, lasting 15 minutes, so the available E must fall during this time and the free A rise for the same period. There is, of course, no evidence that CA represents a hexose-phosphate, but if so we would expect the breakdown of this that is actually observed on tetanization. The further observation, however, that the breakdown continues for an hour or more is not what would be expected on this basis.

Embden and Jost (1928) report a very rapid breakdown of lactacidogen on contraction and reformation on relaxation in muscle. Labile-phosphate in nerve also breaks down on stimulation. In muscle, Nachmannsohn (1928) found a rapid breakdown of creatin-phosphate and a resynthesis, during seconds, accompanying contraction and recovery. We have not been able to demonstrate similar rapid changes in either the acid-labile or the acid-stable phosphates of nerves. Even when nerves were frozen in liquid air while being stimulated, there were no greater changes than have been reported. We cannot conclude from the present results that PO_i is the hypothetical "A", but indications are strong that it does play a significant rôle in nerve function, both in combination with hexose and with creatin.

d. Relation to carbohydrate changes. This is discussed more fully in a preceding paper (Holmes, Holmes and Gerard, 1929) but one point is worth reiterating. In rabbit nerve standing in nitrogen about 5 mgm. per cent of the stable phosphate is broken down. If this be a hexose-monophosphate it represents the freeing of about 30 mgm. per cent of hexose, or an equivalent of lactic acid, if a diphosphate the freeing of 15 mgm. per cent. Actually under similar conditions rabbit nerves were found to form 36 mgm. per cent of lactic acid and lost reducing power corresponding to 37 mgm. per cent of free sugar.

If the lactic acid is formed by the reaction: 2 hexose-monophosphate→ 2 lactic acid + hexose-diphosphate, as in the case of muscle extracts (Meyerhof, 1926), then the fall in sugar would agree with the rise in lactic acid as found, but no loss of stable phosphate should result. If hexose-monophosphate breaks down directly to lactic acid and inorganic phos-

phate, the increase in lactic acid and decrease in acid-stable phosphate should be just as observed, but then the apparent fall in free sugar should be only two-thirds as great. (The hexose in lactacidogen reduces as $\frac{2}{3}$ of a molecule of free glucose) (Embden and Jost, 1928). Despite the excellent agreement in each case, there does not appear any means of reconciling all three observations, and either the phosphate or sugar change must have some other significance. This has been fully considered in the paper previously referred to.

SUMMARY

The soluble phosphate content and its distribution between inorganic, acid-labile and acid-stable forms has been determined in the sciatic nerves of rabbits and several species of frogs.

The content of fresh nerve is variable with individuals, species and time of year. Rest in oxygen leads to slight increase of combined phosphate at the expense of the inorganic.

Rest in nitrogen causes a partial breakdown of both combined fractions to the inorganic form, and subsequent rest in oxygen restores the original condition. The total soluble phosphate tends to increase in nerves kept in oxygen and still more when kept in nitrogen or stimulated.

Tetanization in the presence of oxygen causes the same changes as asphyxia but less completely.

All these changes are progressive for a few hours.

The relations of these findings to the carbohydrate metabolism of nerve, to phosphatide breakdown and to equilibration are considered.

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THE INTERPRETATION OF THE DIPHASIC VENTRICULAR STRIP ELECTROGRAM

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A previous paper described an approximate analysis of the diphasic electrogram from turtle ventricular strips into two causative monophasic electrograms. This analysis demonstrated qualitatively that the recorded diphasic electrogram could be analyzed to give the form of the normal monophasic action potential-time curve. Such a monophasic curve shows a steep rise immediately following stimulation, a plateau maintained at a nearly constant level for a considerable length of time, and a final recovery phase during which the curve approaches the base line asymptotically. The general shape of the curve was essentially that found by Burdon-Sanderson and Page (1880) and had a duration of the order of recorded "monophasic" records reported by several authors since that time.

In order to make possible a future presentation of changes which may occur in the recorded electrogram in response to experimentally determined conditions, it seems desirable to present a somewhat more detailed study of the diphasic electrogram as it may be recorded from different points on the strip preparation.

Considerations to be taken up in this paper concern the form of the rising and falling phases of the R-wave as obtained from different leads on a moist strip suspended in air, the significance of the R-T interval, and finally the form and time relations of the T-wave. Furthermore the appearance in the initial complex of negative waves (Q- and S-waves) and of irregularities in the positive wave (R-wave) seems to warrant careful analysis. These complications have been used by Craib (1927) as direct evidence of the nature of the local electrical circuits within the muscle fibers. Our evidence indicates that they are expressions of what may be included under the general heading of "extrinsic effects" and it is as such that they are interpreted below.

METHODS. The strips used in this series of experiments were nearly all from ventricles of the slider turtle, Pseudemys concinna. A few strips from P. elegans were used and showed little if any difference from those of

P. concinna. The turtle was pithed, the heart exposed, cut from the body, and laid on a glass plate. The ventricle was firmly seized at the base with a pair of forceps. Two cuts with scissors then usually sufficed to remove a wedge-shaped portion from the ventricle leaving the strip to be used lying on the glass plate. Strips treated only with the Ringer's solution used (Bayliss' formula) rarely became spontaneously rhythmic during the course of any experiment. Occasionally a strip became rhythmic a few minutes after preparation. In such cases, this rhythmicity was almost invariably stopped by cutting away a small remaining bit of auricular or funnel tissue.

Silk threads were carefully attached to the epicardium and a minimum of myocardium at the edge of the strip, usually at the two lateral angles and

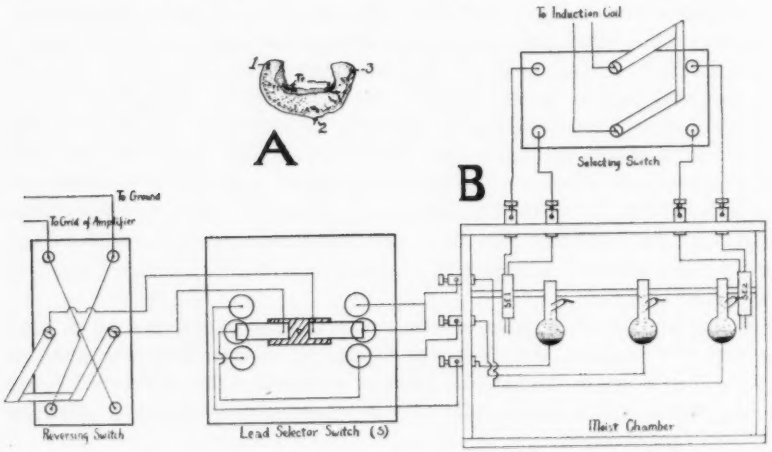

Fig. 1. A. Diagram of ventricular strip showing usual points of attachment for lead-off electrodes. B. Diagram of connections for stimulating and lead-off circuits.

at the base of the frenum, and by these three threads the strip was attached to worsted wicks of calomel electrodes. Leads were thus made as indicated by points *1*, *2* and *3* of figure 1A. These lead-off electrodes were connected through a six-point, spring leaf switch (fig. 1B, *S*) and a reversing switch to the amplifier and recording instrument.

At points *1* and *3* (on the lateral angles of the strip) were placed pairs of stimulating electrodes (*SE1* and *SE2*) connected through a selecting switch with an induction coil. Each pair of stimulating electrodes was placed as nearly as possible symmetrically with the adjacent lead-off point and with the stimulating electrodes a millimeter or a millimeter and a half on

either side of the lead-off point. Stimulation with threshold shocks occurred, therefore, close to the lead-off point and was in some preparations essentially at that electrode. The symmetrical placing of the stimulating electrodes with respect to the lead-off electrode results in a relatively small shock escape to the recording apparatus as was pointed out by Bishop and Gilson (1927). As attachments to the recording apparatus were made to binding posts on the outside of a small moist chamber, after arranging the preparation and closing the chamber by glass plates, no further attention to the strip was usually necessary.

The switches in the circuits made possible the prompt change of leads and points stimulated so that one could obtain, in rapid succession, records from all six of the possible leads through the three electrodes used two at a time, the strip being stimulated at one or the other end and always with the ground lead made proximal to the grid lead.

Stimulation was controlled by a hand key. If the strip had been resting it was stimulated three times at intervals of approximately three seconds, the first recorded reaction being the fourth.

Time intervals of $\frac{1}{5}$ second are marked on the records by means of a Jacquet chronograph suitably arranged.

As recording instruments, a capillary electrometer or a Braun tube (cathode ray oscillograph) were used. With the former, one panel of amplification was used, the circuit in some cases being the balanced circuit formerly described (Gilson, 1927) and in other cases a simple capacity-resistance coupled amplifier. With the Braun tube, the partly shunted output from a two-panel amplifier was used. The Braun tube arrangement used in this work was one assembled by Dr. G. H. Bishop and is a somewhat modified form of the apparatus described by Gasser and Erlanger (1922). Doctor Bishop's apparatus is to be described in a forthcoming publication but the point which made it especially suitable for the work was the introduction first of a shield-plate thermionic tube used to charge a condenser and giving a nearly linear movement of the spot with respect to time, secondly of a two-circuit, oil-damped, spring switch which served to start the motion of the spot along the abscissa and, at a given time thereafter to break the primary circuit of the stimulating coil, thereby stimulating the strip.

ANALYSIS OF A DIAGRAMMATIC NORMAL DIPHASIC ELECTROGRAM. The analysis of strip electrograms as actually recorded will be made simpler if we may first develop a purely diagrammatic electrogram. Assume that the diphasic record obtained is the graphic sum of two monophasic curves, one positive and one negative, but having the same form and with their starting points separated by a time interval equal to the time necessary for conduction through the strip. In the analysis which follows, it is assumed that the form of the local monophasic curve is the same at any point along

the strip from which leads may be taken, and that these lead-off points may be either at the point stimulated or at some other point.

Now consider a strip prepared as indicated in figure 2A, having arrangement made for stimulation at point *I* and with leads to the recording mechanism possible from any two of the three points *I*, *II* and *III*. (For the present we disregard extrinsic effects.) If leads be taken from *I* and

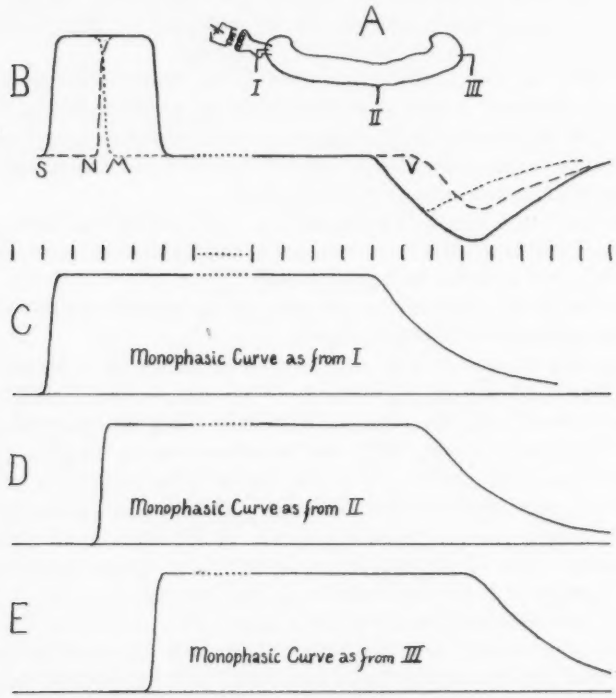

Fig. 2. A. Diagram of strip with leads possible from three points, stimulation at one point. B. Diagrammatic diphasic electrogram from strip with leads at points *I* and *III*, solid line; *I* and *II*, short dashes; *II* and *III*, long dashes. *C*, *D* and *E*. Local monophasic curves as indicated.

III, the resulting diphasic record will show the form indicated by the solid line of figure 2, B. If leads *I* and *II* be used, the R-wave will drop off sooner to reach the base line at the time M. The T-wave will commence at the same time as in the first case but will show an earlier crest. On the other hand, if leads *II* and *III* be used, the R-wave will begin to rise later after a conduction time, *SN*, and the T-wave will commence later at a

time, *V*. The graphic sum of the two half-strip records should give the curve of the whole strip record.

The form of the rising phase of the monophasic curve should be seen in the form of the rising phases of the R-waves of records showing stimulation at the lead-off point or with a conduction interval before the lead-off point is reached. Furthermore, still disregarding the matter of extrinsic effects and multiple pathways, the falling phases of the R-waves of the different records should also have the same form (but inverted) as the rising phases, for the falling phase merely represents a return to the base line due to the activity reaction of the tissue under the distal electrode.

If the monophasic plateau be nearly flat until the onset of recovery, the start of the T-wave should be a sign of the beginning of recovery from the electrical negativity of activity at the proximal electrode.

The time elapsing between excitation at the proximal electrode and the beginning of the T-wave should therefore be in no wise a direct measure of conduction time but merely of the beginning of the dropping off of the monophasic curve as it would (hypothetically) be recorded at the proximal electrode.

After the start of the T-wave, for a period equal to the conduction time through the strip, the T-wave should follow the curve of recovery of the proximal monophasic curve. After that, with recovery occurring at the distal electrode also, the curve should represent the graphic sum of the proximal and distal recovery curves, these two being separated along the time axis by an amount corresponding to the conduction time. As long as the proximal monophasic curve is falling faster than the distal, i.e., as long as the slope of the proximal curve is the greater, the T-wave will continue to have a negative slope. When the slopes are equal, the T-wave will reach its peak and at this point the proximal and distal monophasic curves will probably show opposite signs of curvature. Then, as the monophasic curves approach the base line, asymptotically but with the distal curve always having a slightly greater slope than the proximal, the T-wave will approch the base line and a resting condition. It will be seen that unless the recovery phase of the monophasic record follows a curve which is always concave upward and with a continually decreasing slope, the time interval from the start of the T-wave to its crest will not necessarily be equal to the time required for conduction through the strip. (In the case of the R-wave of a fairly long strip, there is a relatively fast movement from a nearly flat base line, so that measurement from the beginning of rise to the beginning of fall gives a fairly accurate method of measuring conduction time.) It should be noticed that the shape of the T-wave is indicative of the form of the recovery phase of the monophasic curve. A sharp high T-wave indicates a steep slope to the first part of the monophasic recovery phase, whereas a low broad T-wave indicates a more gradual beginning of return of the monophasic curve toward the base line.

ANALYSIS OF THE RECORDED MONOPHASIC ELECTROGRAM. To avoid repetition, it may be said that in general the recorded electrograms agree well with the diagrammatic form considered above. Certain points should be considered here in terms of actual records obtained.

All of our records show the R-wave to have a slight concavity upward at the start. In some of our best records this is reduced to a very small part of the rising phase, but we have no records in which it is entirely absent.

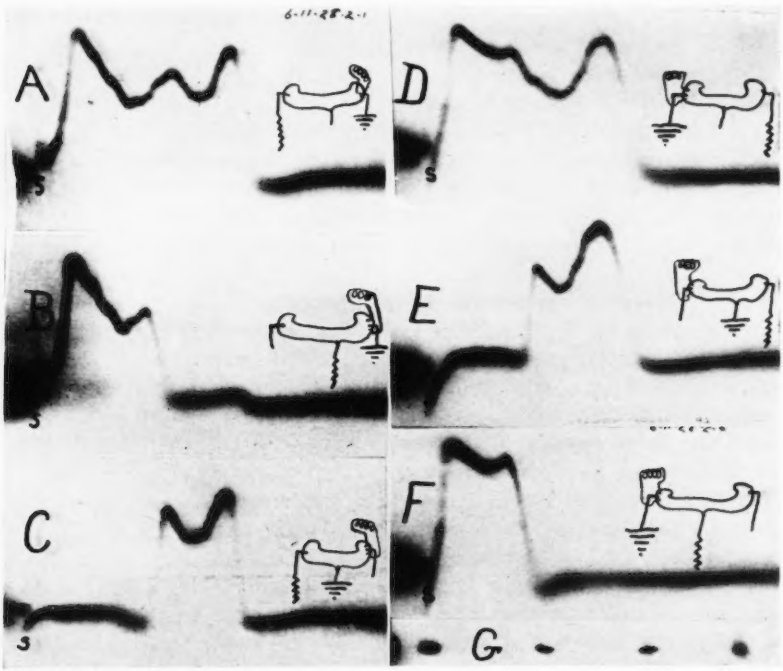

Fig. 3. A to F inclusive. Braun tube records of the initial complex of the diphasic electrogram from strip with stimulus and leads as indicated. G. Time intervals of $\frac{1}{5}$ second.

The time to maximum negativity at 25° averages about 0.06 second. Slight injury of the tissue will cause pronounced slowing of the rising phase. The rising time is essentially the same whether measured at the site of stimulation by immediate response to an induction shock or after a measurable conduction period.

The duration of the falling phase of the R-wave from a strip preparation is generally slightly longer than the rising phase, but it usually has the

same form as the rising phase inverted. The slight increase which sometimes occurs, amounting to 0.02 to 0.03 second, is probably explicable on a basis of spreading of the impulse as it passed along the strip so that the first effect to be recorded at the distal electrode was due to arrival of the electrical impulse in a fiber bundle not directly under that electrode.

The irregularities in the R-waves (fig. 3) are of a fairly constant form in records obtained from strips prepared in the same way and from P. concinna. It will be seen from the records that these irregularities may account for apparently aberrant Q- and S-waves to be seen in some strip records and not in others although great care may have been taken to avoid injury near the lead-off points. Figures 3A and 3D show the R-wave with two main peaks, a minor peak between these two, and definite notches on either side of the intermediate crest. Under certain conditions, especially when there is a slowed rate of conduction, the middle crest may be reduced or even of negative form so that there appears a deep major notch with minor notches on either side of it. Exploration with leads placed at various points along the strip demonstrated that the "major irregularity" (in fig. 3A the middle peak) was due to events occurring at or very near to the apex. The two prominent minor notches are records of events occurring between the apex and the lateral angles on either side. That is, with leads attached on either side of the apex, but well below the angles, the major irregularity was present but not the minor irregularities. With leads on the apex and one lateral angle (as in fig. 3 C) one minor irregularity is present but the major irregularity (of many records) is like to pass unnoticed in the rising (or falling) phase of the R-wave.

In searching for conditions which would account for these irregularities, it was found that the minor irregularities corresponded in time to the passage of the impulse by points at which large trabeculae turned into the ventricular cavity on the right and left sides of the ventricle respectively. These trabeculae need not be cut in the preparation of the strip, although they usually are, but they seem to cause notches in the electrogram in a manner similar to that by which nerve branches coming from a nerve trunk affect the electroneurogram. The production of notches in the action potential record of nerves by such branches was reported by Bishop, Erlanger and Gasser (1926). They found that such branches acted as leads from the inside of the nerve and then became effective, at either grid or ground electrode according to the resistance balance, by electrolytic conduction over the surface of the moist nerve. As a transmitted impulse passed the points at which branches came off, these secondary leads became effective and the resulting records showed notches which corresponded in time with calculations made later on a basis of measurements of the position of the branches. Evidently in the heart strip preparation, the trabecular bundles in the inner surface may act as secondary leads in a

similar way. It will be argued that smaller trabecular bundles ought also to produce irregularities of the R-wave though perhaps in a less degree. Reference to a clear record such as that of figure 3A will show that this is the case, the R-wave being markedly broken by irregularities which may easily be demonstrated to be due to irregularities in the effective potential difference between the lead-off points and not to extraneous difficulties such as vibration of the recording mechanism.

The situation to be considered at the apex is slightly different from that discussed above. At and near the apex, there is a general turning in of the fiber bundles which pass down over the surface of the heart giving rise to the so-called vortex. Furthermore there is at the ventricular apex a small cone-shaped plug of connective tissue. The apex of this cone is directed inward, so that with respect to a lead-off electrode attached to the frenum, an impulse passing down over the surface of the ventricle comes to the base of this cone, spirals up along its side, thence passes down along the distal side, and on away. At the apex, therefore as at the larger trabeculae, the sudden inturning of the muscle fiber bundles would produce irregularities of the potential record as the impulse passed this region.

However the complications of the simplest possible pathway are of themselves such as to introduce irregularities in the record. Besides the irregularities of the R-wave due to localised peculiarities of the strip, there is to be noted, invariably in relatively long strips, a depression between the two major peaks of the R-wave. The interpretation of this as due to a shunting effect between the leads will be treated of in the discussion.

Following the R-T interval during which nearly zero potential exists between the leads, there occurs the T-wave which is a sign of electrical recovery successively at the two leads. The T-wave, as recorded from strips, follows almost exactly the diagrammatic curve described in section III and at 25°C. begins about 1.5 seconds after stimulation at the proximal electrode. We have plotted the T-waves of a number of Braun tube records and find that, after its first inflection, the first phase of the T-wave approximates the course of a logarithmic curve. It is this convenient curve which has been extrapolated in plotting the electrical recovery phase of the monophasic curve.

It was shown in section III that if our interpretation of the electrogram is correct, the form of the T-wave is determined by the recovery phases of the two monophasic curves. This point is supported by the records which we have obtained. Our records also show that, with a strip preparation, the form of the T-wave is a fairly good index to the general condition of the tissue. A strip which is fresh gives a fairly high, steep T-wave whereas one which has been severely fatigued gives a low broad T-wave.

That the T-wave is dependent for its appearance upon the successive

subsidence of processes under *both* effective electrodes is manifest from records such as the capillary electrometer record shown as figure 4G. The diphasic record from a similar but normally conducting strip is shown in figure 4A, in which there is to be seen the escape from the break shock used as stimulus, the double-peaked R-wave, and the usual T-wave. The strip from which the record of figure 4G was obtained developed a functional block at the apical region so that conduction could occur through either half of the strip but not from one half to the other. The lead-off electrodes were placed one on either lateral angle, stimulation occurring at one of these (the right). As will be seen from the record, the response of the proximal half of the strip is recorded as a monophasic curve lasting approximately the time necessary for excitation to spread to the apex. There is no T-wave because there is no residual negativity at the distal electrode when recovery has commenced at the proximal electrode. The effective lead from the apex to proximal and distal electrodes being the same, the apex does not act sufficiently as an effective distal lead to the grid electrode to have any effect in the production of a T-wave. However, the monophasic record obtained in such a case is not as prolonged as the true monophasic curve of local response but is essentially a diphasic curve in which the final phase (T-wave) is suppressed. Had the distal electrode been placed close to the apex, a T-wave would have appeared in the record.

Discussion. The detailed analysis of the diphasic electrogram reported above has been undertaken to demonstrate the various ways in which the strip electrogram may be used to give normal values with which there may be compared values from electrograms of strips subjected to experimental changes. The records published were from strips showing a minimum of injury effects at the leads. The strip preparation had been selected previously as offering a heart muscle preparation with relatively parallel fiber arrangement. The stimulus was applied at a known point, in many cases at the proximal lead-off point.

Despite all these attempts to keep conditions as simple as possible, the problem of the electrogram from heart strips remains complicated. Leads must be attached to make shifting of the lead-off points impossible and attachment frequently means injury. To consider the heart strip literally as offering a parallel fibered arrangement is convenient but demands overlooking the spiral arrangement of the fibers in the ventricular wall, the apical vortex, and the frequent turning in of fiber bundles to form trabeculae which normally project into the cavity of the ventricle.

The fact that the heart strip is electrically conductive means that as the impulse moves from the proximal electrode toward the distal, the leads are shunted by the surface of the strip itself and the recorded (*relative*) electrical negativity of the proximal lead-off point becomes less as the impulse moves toward the distal lead. This shunting takes place especially over dead or

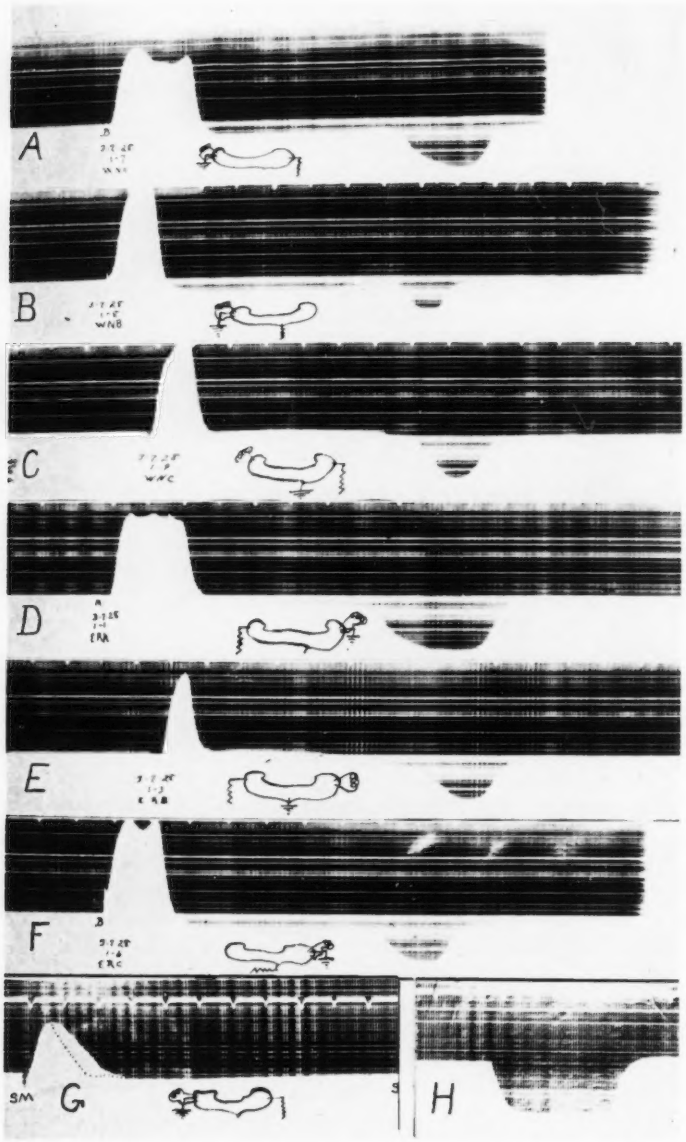

Fig. 4. A, B, C, D, E, F. Uncorrected capillary records of strip with leads and stimulus as indicated. G. Capillary record from strip with functional block at apical region. SM, stimulus escape. H. Normal curve for capillary used.

inactive regions of the strip. However, as the impulse approaches close to the distal lead, lines of current ahead of the impulse result in making the tissue surface at the distal lead-off point relatively more positive. Thus a record shows the depression of the R-wave following the first major peak but leading up to a second peak. With the arrival of the impulse at either electrode the local reaction becomes so much more effective than the various extrinsic circuits that the latter become much less apparent. Figure 4G offers a good demonstration of the first part of the major R-wave depression, the impulse being extinguished at the apex so that the second peak, seen in figure 4A, is not present. Bishop and Gilson (1929) have recently dealt with the irregularities which may develop in the electrogram from the frog's sartorius mucles under various conditions of immersion in a saline bath with the lead-off electrodes placed 1, on the reacting muscle fibers; 2, on inactive muscle fibers near active fibers; 3, at various points in the saline bath. Their discussion of the experimental results should be referred to as it can be carried over almost intact for use in explaining the simpler of the irregularities found in the heart strip electrogram and such discussion will therefore be omitted here. Their analysis of waves which may be interpreted as "Q-waves" and "S-waves" caused by external shunting circuits over a moist muscle in simple relation to the demands of Ohm's law, is especially pertinent in view of Craib's (1928) interpretation of his own excellent experimental results on heart strips and skeletal muscle, discussed by Bishop and Gilson in the latter paper. It seems well to point out one fact in this paper, however. One of the demands of Craib's hypothesis is that in a heart strip immersed in saline (and a living strip kept wet with Ringer's solution we believe should come into the same category) an impulse conducted *to* the *proximal* electrode should develop an R-wave which is preceded by a Q-wave. Further, an impulse conducted *beyond* the *distal* electrode toward the end of the strip should show the R-wave followed by an S-wave. As will be seen from the Braun tube records published, there may or may not be Q- or S-waves present under those conditions.

In no case of the records published was the distal electrode at the end of the conducting pathway. It happens that these records were made with leads placed so that the irregular surface shunt effects were relatively slight. However, if the leads be deliberately shifted about in search of the maximum effect of irregularities, definite Q- and S-wave appear (cf. Bishop, Erlanger and Gasser, 1926). We have even obtained records with a positive (upward directed) Q-wave preceding the R-wave (also upward directed). It will be realized that a complete explanation of the irregularities seen in records from a moist strip must explain not only the presence of the irregularities but their absence from many records obtained from similar strips but with differently placed leads. If now, as was done by

Craib, a strip be placed in a saline bath and lead electrodes be placed not on but "near" the strip, the possibilities for external circuits are enhanced and supernumerary waves appear, as Craib finds them. These are not necessarily due to the doublet nature of the microscopic muscle units (though such, with qualifications, may be their nature) but may be explained as above. Technically, we have not as Katz (1928) reported "failed to confirm Craib's findings" as we have not completely repeated Craib's experiments under conditions identical with those he used. We do indeed confirm many of his experimental findings and realize the validity of others. It is in the interpretation of these results as evidence concerning the unitary electrical reaction of heart muscle that we differ.

Certain features of the electrogram analysis may be emphasized here. For example, the extrinsic lead effects in a strip preparation, though they may be noticeably present, mask only to a slight extent the changes occurring under the lead-off electrodes and interfere to an even less degree if only time relations without regard to height of the excursion be taken. Since the extrinsic effects which do occur are quite constant, they may be identified by the use of a method such as described above, namely, the arrangement of three leads and two stimulating electrodes so that various combinations may be selected.

The time relations of the R-wave, which occurs as an expression of rather rapid and constant electrical change, are quite constant in different "normal" records. The T-wave, on the other hand shows much more marked changes of form in response to slight effects of unequal conditions at the leads as a result of local injury or depression. It should be clearly recognized that although the beginning of the T-wave in uninjured, nonfatigued strips occurs at a definite time, the introduction of fatigue or injury causes very pronounced changes to be seen. It is to avoid misconception on this point that we have throughout this paper persistently used the word *normal* although even the normality of the strips used must be regarded as relative. However, changes in the form of the T-wave may be interpreted as due to changes in the form of the recovery phase of the monophasic curve and are being studied from that standpoint at the present time. Certain definite changes which can be produced experimentally in the T-wave will be described in a later paper. It is changes of this sort which make it so difficult to localize any corresponding feature of the cardiac cycle, such as the "end of systole" or the end of the absolute refractory phase in terms of the T-wave of the diphasic electrogram. In the case of the determination of the end of systole the difficulty is largely one of devising proper mechanical registration. With respect to the end of the absolute refractory phase, there is fairly general agreement that normally the absolute refractoriness is ended at some time shortly after the T-wave has reached its crest. Since the crest of the T-wave is determined

by the forms of the recovery phases of the component monophasic curves it is possible to say that absolute refractoriness is ended at some time shortly after the beginning of the monophasic recovery phase. Records are reported in which a second effective stimulus has been sent into a heart preparation before the crest of the T-wave (see Katz, 1928) but we know of no case in which a second stimulus has been reported as effective before the *start* of the T-wave, that is to say, before the monophasic curve has begun a definite return toward the base line. Whether the end of the absolute refractory phase can be accurately located in terms of the monophasic curve remains a matter for further study. Certainly the work of Lewis and Drury (1926) on the apparent and true absolute refractory phases indicates that further work of this sort must give careful consideration to the differences between responses as they occur at the point of stimulation and as they may be observed in the usual manner at some point to which conduction occurs.

It should be noted that the monophasic curve obtained by analysis of the diphasic record was derived by starting with the beginning of the electrical response at the point of stimulation (at one lateral angle of the base). That such analysis applied directly to other points along the strip which we have examined as a transmitted impulse passes them, must be regarded as good evidence for the validity of the basic assumption made, namely:— That the duration of the local response from the turtle ventricular strip is the same at all points along the strip in uninjured tissue. If in the turtle ventricle, the durations of the local responses were different, as for example between apex and base, there would be a marked difference between the forms of the T-waves as recorded when the distal lead-off electrode was at the basal angle and when it was at the apex. The actual T-waves recorded under such conditions, however, show differences in T-wave form which may be interpreted as due only to differences in the length of the strip between leads.

At the present time we find no reason for any considerable revision of the general description of the local monophasic action potential curve given in our previous paper (Gilson, 1927). Certain implications of that paper concerning the simplicity of the heart strip preparation should be modified. The heart strip is simple only in comparison with less simple preparations. Some of the complications involved have been referred to above and there are many others. The strip preparation is, however, simple with respect to the entire heart and we believe that it is only through careful study of a preparation offering relatively simple complications that a proper understanding of such intricacies as those involved in the clinical electrocardiogram will be made possible.

SUMMARY

1. The analysis of the "normal" diphasic strip electrogram from the turtle ventricle has been undertaken in a manner more quantitative than has previously been possible.

2. This analysis shows a close agreement of records obtained by various leads with those derived by graphical summation of the hypothetical, normal monophasic curves obtained according to the method previously described.

3. That the curves from various points along a strip are amenable to the same analysis indicates that, in the normal turtle ventricle, the electrical response to stimulation is the same at all points, both as regards potential developed and the duration of the electrical change.

4. Irregularities of the R-wave as recorded by the Braun tube are shown to be due to anatomical irregularities of the tissue itself and also to shunting effects especially over the inactive tissue between the lead-off points.

5. The start of the normal diphasic T-wave is shown to be due to the beginning of electrical recovery at the proximal lead-off point. Other features of the T-wave are discussed.

I wish to express my appreciation of Doctor Bishop's assistance and advice in the preparation of this paper.

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ACTION POTENTIALS FROM SKELETAL MUSCLE

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In recording the potentials from skeletal muscle when only a few of the fibers are stimulated, it is found that the form of the potential varies, depending upon the position of the electrodes on the muscle surface with respect to the active fibers (plate 1). The record of the potential, when one electrode is placed over active fibers and the other on the opposite side of the muscle, at about the same distance from the stimulating electrodes (plate 1, *d*) appears similar to certain records published by Craib (1928). These records appear capable of analysis in accordance with the conventional core conductor-membrane hypothesis particularly developed by Hermann, Bernstein and Lillie.

In recent papers Craib (1927, 1928) has advanced the thesis that muscle immersed in a fluid conducting medium produces an electric field when active, *as if* this activity involved the passage along the muscle surface of axially oriented doublets or dipoles, the first positive pole ahead of the negative. There can be no question of the validity of this statement in so far as a dipole connotes merely a difference of potential. The core conductor or membrane theory of Bernstein involves such axially oriented potential differences. These result from a primary alteration, taking place with activity, in the potential difference of a *transverse* polarization "doublet," usually referred to as a Helmholtz double layer.

According to the core conductor theory, current will flow from the highly polarized region on either side of a depolarized active region, partially depolarizing the adjacent regions. The membrane hypothesis as conventionally diagrammed for nerve and muscle indicates a flow of current along the outside of the fiber from anode to cathode and back through the core from (external) cathode to anode (Bernstein, 1912; Hermann, 1879); Lillie (1926) in particular has developed this theory in a manner that employs the idea of these "eddy currents" as stimuli to adjacent regions, and Pond (1921) has studied the properties of muscle as affected by the conductivity of the surrounding medium. Such currents in nerve have been investigated in this laboratory (Erlanger, Bishop and Gasser, 1926). The two hypotheses, therefore, envisage the same facts, a longitudinal difference of potential outside the fiber, but Craib's

hypothesis as so far stated ignores the primary site of origin of the potentials.

To explain recorded curves of action potentials, however, Craib employs a further assumption regarding the arrangement of the potential forces. In discussing skeletal muscle (1928, p. 57, 58) he argues that current cannot flow from the positive pole of a doublet axially oriented along a muscle strip ahead of this doublet and back to the negative pole, without an *appreciably thick* layer of *external* conductor. He appears not to have dealt with the case when, at or just above threshold, the few active fibers have a return circuit through the rest of the muscle. This argument involves that the essential battery or source of potential of the muscle be directed longitudinally along the axis of the fiber, not transversely across

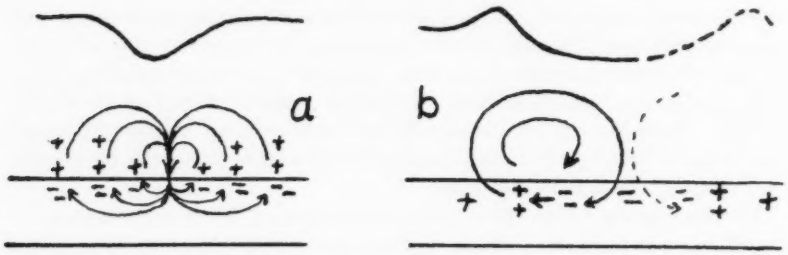

Fig. 1. a. Diagram to illustrate core conductor theory, active region of surface negative to all other points of surface. Source of current is polarized membrane of unexcited regions acting as a battery. See text.

b. Diagram to illustrate a dipole theory in which the source of current is a longitudinally directed potential difference in the core, the current flowing through the surface as a passive structure.

the surface. The muscle membrane then would constitute a resistance in the *external* circuit of the source of potential, instead of being a constituent of the battery itself. The current of "activity" or action current then must flow from a source in the core, through the surface ahead of the excited point, back along the surface, and into the core at the active region. Any area of the surface closer to the active region than another area must have more current flowing across it, and be made relatively more positive, to any further region, with an adequate external medium surrounding the fiber. Any cross section of a fiber would be effectively an equipotential one, instead of the surface being actively polarized transversely.

Craib finds experimentally that potentials recorded from immersed or partially immersed muscles have a polyphasic character, and to explain these curves, he argues that the first phase is produced by an *elevation*

above the resting level, (more +) of the potential at the surface of the muscle, ahead of the advancing wave of negativity. On the core conductor theory for the simple case of a muscle symmetrically surrounded by a conducting medium this region should be *relatively* positive to the active region to be sure, but relatively *negative* to regions further ahead of the excitation.

Craib's theory involves in effect that figure 1*a* is incorrect, figure 1*b* is the true condition for muscle in a conducting medium, as pictured in potential differences and current flow, near the site of activity (Craib, 1927, fig. 16) the poles of the dipole being separated by a very short distance.

These two diagrams differ as to the site of the potential forces, and the question arises whether the experimental findings can be adequately interpreted on the basis of the first diagram without the assumptions involved in the second. Not only do Craib's experimental findings not controvert the conventional theory, but we believe they offer the strongest evidence in its support. We propose to argue (section 3, below) that his results can be predicted from theoretical considerations, if one deals adequately with the consequences of the well known assumptions involved in the conventional core conductor membrane theory. A failure to obtain the results he did obtain would be the first telling blow received by the membrane theory since its inception. We hold no brief for this theory, and do not claim finality for our application of it, but we have not yet been able to find anything in nerve or muscle which is inconsistent with its implications.

1. *Complicated diphasic records from muscle due to asymmetrical leads.* We may first describe the conditions giving rise to complicated records, in the usual uncritical terminology of potential changes presumed to develop at the surfaces of active tissue, disregarding the ultimate source of the potentials. The first "triphasic" potentials to be accounted for in Craib's records and ours are recorded from electrodes, one closely adjacent to the muscle surface, which we may call the adjacent electrode, and one distant in the conductive environment. Curve *h* plate 1 (Craib, 1928) is quoted as typical of this set of experiments. The sartorious muscle, instead of being immersed in a fluid medium, is in place in the body, with its distal end dissected free and stimulated, the leads being one on the muscle and the other (in effect) from the body. The added salt solution appears to be an immaterial detail.

Bishop and Gilson (1927, fig. 1 *A*) discuss such a lead.[1] The string

[1] To record the variation at *only one electrode* (Craib, 1928, p. 55), "the method that suggests itself . . . . is to place one electrode on the tissue and the other at a distance . . . . sufficient to eliminate any material change in the potential at the distant electrode in consequence of tissue activity." The context suggests

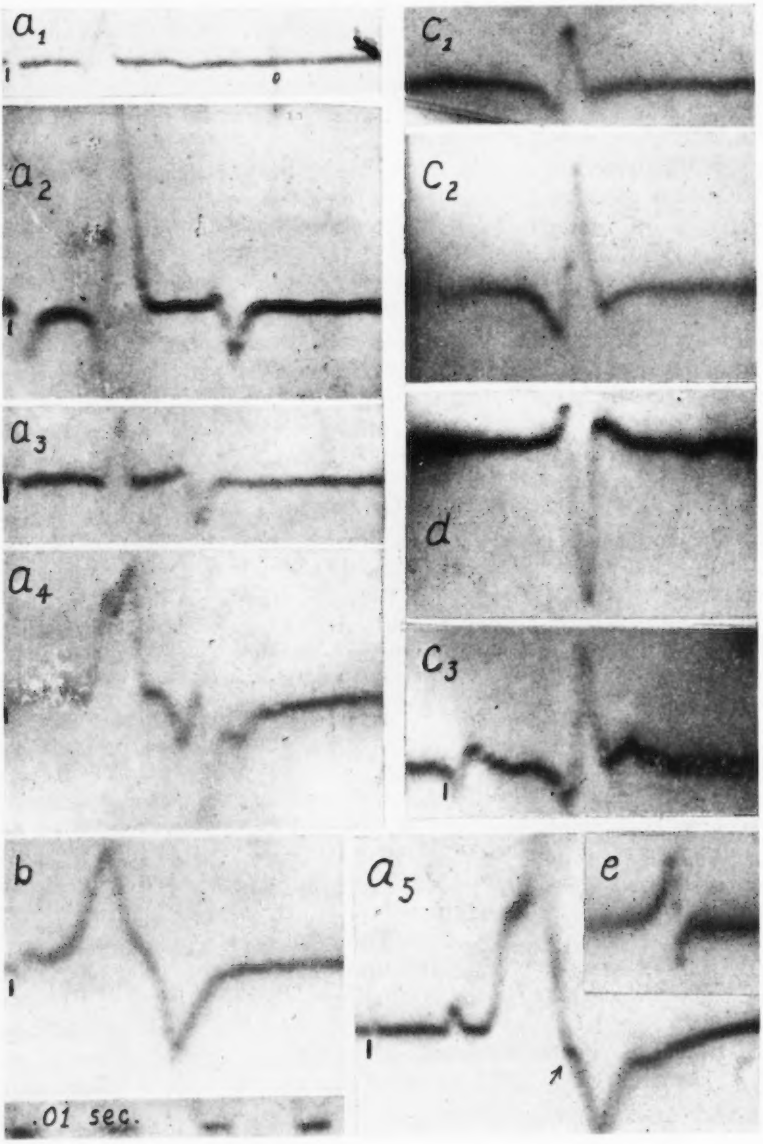

Plate 1

Plate 1. a_1. Threshold response of green frog sartorius, in air, m cathode, sensitivity 50 mm./m.v. k is slightly off active fiber path. (See diagram a, figure A, and corresponding diagrams for b, c, etc.)

a_2. Same arrangement, stronger stimulus, not all fibers conducted to k, which however was over active pathway.

a_3. Same as a_2, k moved toward j, approximately on active pathway, threshold.

a_4. Same, stronger stimulus, two or more groups of fibers of different conduction rates stimulated at m, separate out further at k than at j; 25 mm./m.v.

b. Current reversed, stimulus at n, leads as before.

Stimuli for above, 3 mm. from uninjured pelvic end, induction shocks, electrodes j and k placed against edge of muscle, slightly adjusted between records, distance m to j all records except a_2, 12 mm.; j to k 12 mm.; k to end of muscle 10 mm. Isometric. For a_1 and a_3 there was no visible mechanical response; for a_2 a just visible reflection of light from the muscle surface; for a_4 and b just visible movement of the muscle as a whole, shock record marked with ink line. Time below in 0.01 second. T = 23°C.

a_5. Same arrangement as a_4, opposite sartorius muscle, but stronger stimulus, sensitivity 10 mm./m.v.

When the muscle is stimulated on one side only, and at just above threshold, a, a triphasic record is obtained at each lead provided the lead is precisely over active fibers. If slightly off the active pathway, the record is simple. The "positive" phase is due to shunting of active fibers by inactive as explained in text. When more fibers are stimulated, a_5, this shunting is not effective, except that the small first process travelling ahead of the main electrical wave, finding the adjacent fibers not yet active, is shunted asymmetrically and thus becomes triphasic. No sign of positivity is discernible in the main process, even the suggestion of it at the start of the second phase (marked with arrow), being due to the first maximum of a triple negative wave at the second electrode, the three parts separating out with conduction.

c_1. Sartorius muscle partially immersed in Ringer's solution, lead j from center of air-exposed surface, lead k from wall of container *via* bath. Stimulus 2 mm. from pelvic end, about threshold response, 50 mm./m.v.

c_2. Slightly stronger stimulus, two fiber groups stimulated which have slightly different conduction rates. Mechanical responses not visible to the eye; it is not known how many fibers are being stimulated. The tripolar stimulus localizes the response under m, and n'' may be moved to balance out the stimulus record.

d. Sartorius in air, stimulus at m, leads on opposite edges of muscle, at middle region, just above threshold response. Both leads record the impulse nearly simultaneously, the diffuse lead j (opposite side of muscle from active fibers) giving a record that lasts longer than that from the localized lead k. Added to this is the effect of asymmetrical shunting by inactive fibers. The record is in a sense the

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that Craib believes he can disregard the change of potential along the interface between muscle and the external medium or the body, as the excitation progresses along the muscle. In a former paper we have stated (1927, p. 487) that under similar circumstances in the body, "the (monophasic) action potential is recorded as the impulse passes the electrode, though before or after this passage, small waves of opposite sign may be recorded at other effective points of contact of the muscle with the body (fig. 1 *A*). The action potential is monophasic in general . . . . except for secondary polyphasity as noted above." We should perhaps have stated, "The action potential is diphasic, but one phase is much more prominent than the other."

algebraic sum of the first phases of the diphasic records a_2 and b, affecting the recording instrument in opposite directions, so that the effect of the diffuse lead (record b) adds to the "positive" effect of the localized lead (record a_2.).

c_3. Leads as c_1, different muscle, stronger stimulus, lower sensitivity, partially immersed muscle. When the lead j is moved off the active pathway from m, the record is simply diphasic. When the stimulus is then made stronger to reach the fibers lying under the now displaced electrode j, the record becomes again "triphasic" due to asymmetrical shunting.

e. Sartorius immersed in Ringer's solution, leads from end and from bath. Diphasic record, slow rising phase at first lead (bath) as impulse travels toward second lead along the bath-muscle contact; sudden reversal as it enters the tubular electrode. Just above threshold, 50 mm./m.v.

When two leads are placed across the muscle as in the above experiments, there should *theoretically* be a physiological cathode under either electrode, and impulses should arise at both edges of the muscle. This is usually not the case in our experience, provided both stimulating electrodes make equally narrow contact with the muscle, and when the stimulus does arise under the anode at a lower strength of shock than affects the tissue under the cathode, a reversal of effects follows wetting the muscle at the cathode. Presumably cathode and anode at the exterior of the muscle polarize differently. The conditions obtaining can be observed when the stimulus causes a visible mechanical response, and it is assumed that weaker stimuli act at the same electrode as stronger ones.

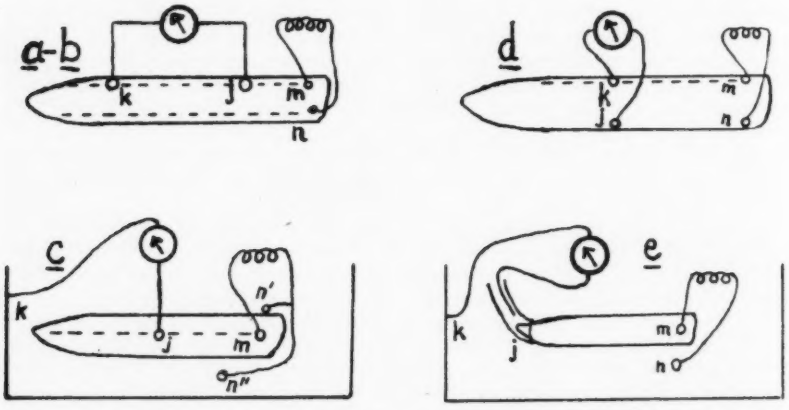

Fig. A. Diagrams of stimuli and leads for records of plate I, q.v.

galvanometer does not do justice to the complexity of the resulting records. As the (relative) negativity propagated along the lifted portion of the muscle reaches the edge of the contact with the underlying body, part of this contact surface becomes negative, which in effect means that the body forms the first lead reached by the impulse. The first deflection is, therefore, assignable to a negative effect at the body contact, directed toward the distant electrode, there being an obvious difference of potential be-

tween inactive and active muscle along the body contact, before the impulse reaches the opposite adjacent electrode. This difference of potential remains after the negativity has passed the electrode on the muscle, and thus the effect is a prolonged "positive" deflection which is transiently reversed by a short negative one. The negative potentials, in the usual terminology, are recorded from opposite surfaces of the muscle. There would be other small variations as the excitation passed the point of exit of the blood supply, etc., which the string is possibly not adequate to differentiate.

The negative wave can be eliminated from its interfering middle position in the record according to either theory under discussion, by causing it to arise very late, i.e., by putting the lead which times it at the distal end of the muscle from the stimulus. This is conveniently accomplished for an immersed muscle by drawing the thread, tied to the tendon end of the sartorius, up into an electrode tube until the tendon end of the muscle plugs the electrode aperture more or less tightly. Under these conditions the lead is obviously a diphasic one, in the usual sense. As the propagated active process renders the bath significantly negative to the unexcited muscle near the distal electrode,[2] a deflection occurs, which increases in amplitude with progress of the excitation. The deflection is suddenly reversed when the electrode is reached, as in the ordinary diphasic record with leads closely approximated (plate 1, *e*). There is no dividing line between this diphasic lead and one which gives an apparently triphasic record (as *c*), and the different forms of the two phases are functions of the different contacts with the muscle of the two (effective) electrodes.

Such records are still, however, only rough composite pictures of the activity of a many fibered muscle. Examined near threshold response, in air, the wave is seen to consist of potential components due to dissimilar fibers, somewhat like the potential wave in nerve. With a sensitivity of the amplifier-oscillograph combination of up to fifty millimeters per millivolt, a very few fibers give a well-defined potential, whose duration is considerably shorter than that when all the fibers of the muscle are active (plate 1, *a* and *b*). For instance, with ordinary diphasic leads it is possible to obtain the two phases well separated out in the conduction distance

[2] Craib infers from his own statement of the membrane hypothesis (1927, p. 96) that " . . . . in any system consisting of a muscle mass imbedded in a conducting medium only curves in which the deflection is always on the same side of the base line (or so-called monophasic curves) should result from one lead directly applied to the muscle mass and the other to a point at a distance." This passage and its context ignore the obvious result of any negativity of the muscle contact with the bath. The bath as a whole acting as an electrode lead, will become relatively negative to the inactive region of muscle, due to negativity at one region; but this result follows directly from the usual concepts of potential differences across the muscle surface involved in the membrane hypothesis.

afforded by a green frog sartorius muscle at threshold at 25°C., while the two phases interfere when stronger stimuli are applied. If the muscle were stimulated in the middle this might be due to nerve excitation, but when stimulated within two millimeters of the uninjured pelvic end, components with different conduction rates may be observed to separate out with conduction, a complex wave being recorded from the farther electrode when the nearer electrode records a simple first phase.

If the recording electrodes are located precisely upon the active fibers, it will be apparent that these fibers are shunted by the inactive remainder quite as if they were in contact with an external conducting medium. Each phase then exhibits a positivity preceding and following the main negative deflection, as in Craib's records, and presumably for the same reason. If one or both electrodes are now moved off the active fibers, the

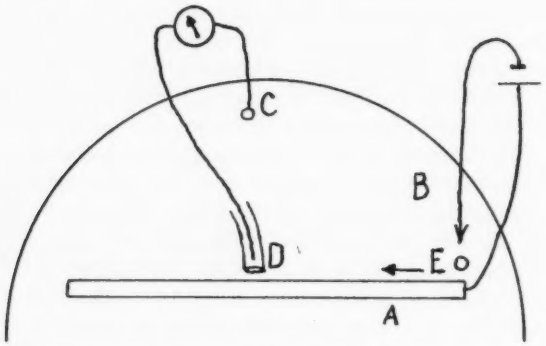

Fig. 2. Diagram of model to illustrate diphasic leads from muscle. See text.

waves recorded from the inactive regions due to adjacent active fibers are no longer "triphasic," but diphasic. The potential changes of the surrounding medium then show no signs of a positivity of the muscle ahead of the active point. This initial positivity is apparently confined to that aspect of the active fibers which is *least* affected by contact with the surrounding inactive ones. The opposite surfaces of the active fibers in contact with air and with muscle apparently differ in their potential changes. The situation corresponds to that of a partially immersed muscle with the electrodes upon the air-exposed surface.

It is thus seen that one triphasic effect may be due to diphasic leads, (one localized and one diffuse), the potential changes summing algebraically; that a second triphasic effect may be due to an asymmetrical shunting of the active muscle fibers. The latter appears when the lead is made from that surface of the muscle which is shunted least, i.e., by the highest

resistance. The first effect does not require a consideration of the internal structure of the muscle, or of the source of its potentials (section 2). The second effect follows as a direct consequence of the conditions assumed by the core-conductor hypothesis, due regard being given to the asymmetrical conditions of the experiment (section 3).

2. *A model for reproducing the fields about a symmetrically immersed active muscle.* The potentials recorded from two electrodes *in the immersion medium* may be illustrated by means of an experimental model which consists of a surface initially equipotential, along which may be moved a point charged negatively with respect to it. A metal strip A is immersed in a dish of salt solution, and connected to the positive pole of a battery. A wire B connected to the negative pole may be moved through the medium. A wire C distant in the medium serves as one lead, and a second wire D enclosed in a glass tube is the localized lead, to a recording instrument. The wire B may now be moved along the "outside" of the "muscle" to imitate effectively, with respect to the surrounding medium, a local depolarization of the surface as the action potential is propagated. As this electrode touches the solution (at E) it is apparent that electrodes C and D are both on a potential gradient from E to A, and that the potential at C has become more negative than that at D. This gives an initial phase in one direction. As the "depolarization wave" passes along A under D there is a deflection in the opposite direction, since now the potential gradient from C to D has been abolished. Thereafter the previous conditions will be reëstablished as the electrode passes away from D. The phenomena are too obvious to require elaboration.

When D is close to the end E, the curve is diphasic, corresponding to the condition Craib describes, that the initial "positivity" becomes prominent as the electrode D is moved further from the point where the muscle enters the fluid, the stimulus being applied to the end of the muscle in air. The model is not quite in its most representative form, however, until we coat the metal with a layer of resistant material, e.g., gelatin, by the localized removal of which areas of metal may be exposed to represent cut trabeculae of the heart strip, or the exit of the blood supply of a muscle, etc., giving a low resistance lead through this "connective tissue" to the bath. Then as the negative lead is moved along the positive surface, an irregularity in the deflection occurs as it passes a bare spot of the metal, giving a polyphasic record.

It is also obvious that the "core" can be dispensed with, quite as the character of the source of potential, in muscle, can be ignored by Craib when considering the currents flowing in the external medium, since what we are dealing with is a negative variation from the resting level, that of the surface initially at the potential of the bath.

3. *The core conductor-membrane theory applied to potentials recorded from*

asymmetrical leads on muscle. We may now examine the implications of the core conductor theory for the condition where a tissue is either submerged, or only partially immersed, one aspect of the tissue being in contact with an extensive layer of conducting fluid. While Craib purports to be discussing the fields *in the medium about* the active muscle, he actually places the aperture of a tubular electrode *close to the surface* of the tissue, and *thus partially insulates this region of the surface from the bath.* The simple considerations that applied to the field about the model described above must now be modified to include the effects of partially or completely insulating the region under one electrode from the bath, as by allowing the

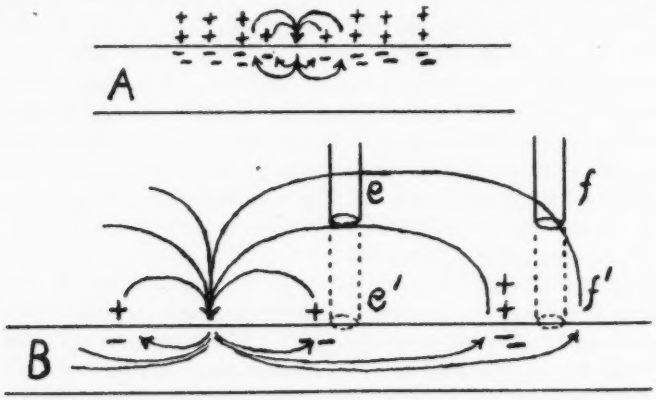

Fig. 3. a. Diagram of muscle fiber acting according to the core conductor-membrane theory, current flow localized by high resistance of muscle surface, surrounded by thin film of solution.

b. Immersed muscle, action currents less localized than in 3 a, electrodes at positions *e* and *f* on external potential gradient through solution, at position *e'* and *f'* effectively on internal potential gradients through the core. Further explanation in text.

muscle surface to lie outside the bath in contact with air, or by more or less closely applying the electrode to the submerged surface of a muscle. It will be shown immediately that the inside of the muscle cell can no longer be disregarded, nor is the arrangement of the potential source with respect to the surface of the fiber without significance. Our figures of plate 1, *c* and *d*, also involve such a lead in the final analysis, a condition which is probably normally present in the heart, as the impulse passes along a pathway asymmetrical with respect to the whole organ.

Figure 3 *A* is a schematic diagram of the condition in one active fiber, when the layer of fluid about it is thin (wet surface, in air) the upper half only of the diagram being completed. The source of current is by assump-

tion the transverse potential difference across the membrane in the "inactive" regions, and these regions adjacent to the "active" point are supposed to be depolarized by current flowing from them, quite as a battery runs down. Activity involves, by assumption again (Bernstein, 1912), increased permeability and consequent discharge of the polarization at the active region, the reason for this not being a pertinent matter. The metabolism of the muscle recharges the battery (Biedermann, 1896). The current returns through the inside of the fiber, the depolarized region tending to be restored by the currents flowing through it (Lillie, 1926). There is thus established a potential gradient from "inactive" to "active" regions outside the fiber, and from "active" to "inactive" inside, due to resistance of the pathway. The currents are effectively localized near the

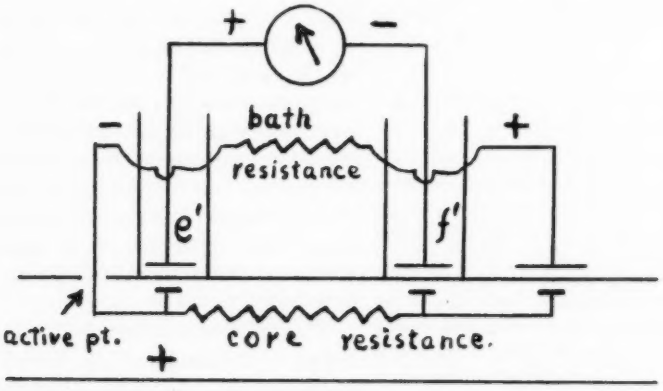

Fig. 4. Diagram of potential forces in active fiber according to the core conductor theory, membrane potentials being represented as batteries.

active region due to the high resistance of the circuit. This condition has been investigated for nerve (Erlanger, Bishop and Gasser, loc. cit.), and the artefactual complications of effective double leads, etc., due to an external conducting pathway along the nerve, have also been discussed (Bishop, Erlanger and Gasser, 1926).

When the resistance of the external circuit is lowered, the conditions are of the same type, except that an appreciable depolarization must extend further from the active region along the as yet inactivated fiber (fig. 3, *B*, immersed tissue). All regions near the active region will be more depolarized than regions further away. An electrode, *e*, brought *near* the fiber but effectively in the surrounding medium will be at a point on a potential gradient inclined toward the active region. Inside the fiber core the potential gradient will be in the opposite direction. The membrane in

the region below this electrode, *e*, will have a smaller potential difference across it than at some other point, *f*, further from the excited region. As the excitation conducts toward the right, past these two electrodes, they will record a diphasic potential change in the usual sense, provided they are on the external potential gradient and not applied closely to the membrane, the first electrode *e* becoming first negative with respect to *f*. Figure *b*, plate 1, shows a record so obtained from a sartorius, in air, the inactive muscle substance serving as the external conducting medium (see legend).

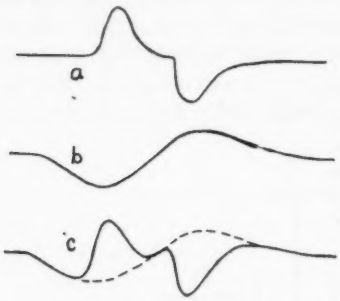

Fig. 5. Diagram of two components of potential led from two leads at the surface of an active muscle fiber, one aspect of which is in contact with a conducting medium as in figures 3 and 4. *a* is potential change due to active depolarization of membranes under each electrode. *b* is potential change in the core of the fiber due to asymmetrical shunting of active potentials by the bath. *c* is composite of two curves as recorded.

If, however, the electrodes are applied closely to the surface, the regions under them are (partially) insulated from the bath, and the electrodes are no longer simply on the external potential gradient. On the contrary, owing to the high resistance of the electrode or galvanometer circuit, this circuit through the electrodes will draw, in general, an insignificant current. The insulated surfaces will tend to remain fully polarized to a potential difference characteristic of the resting activity of the tissue.

This potential difference will exist between the outside of the membrane to which the electrode is applied (or in the whole muscle, the tissues immediately overlying it), and the inside of the membrane which is at the potential of the core. The potential at a given region along the core depends on currents flowing through it which in general return through the bath. However the potential differences led off at two points along the *outside* of the fiber which are insulated from the bath will tend to be the potential differences along the core, and not those in the external medium. This situation is diagrammed in figure 4.

Thus, as the active process passes toward the right (figs. 3 and 4) the first electrode e' (dotted line) will become first positive to f', since the core potential under e' is positive to that under f'. As the active process itself passes e', however, the electrode becomes suddenly negative, due to active depolarization of the membrane beneath the electrode. When the active process passes beyond e', the previous condition is restored, and the whole cycle is repeated at f' as the mirror image of that at e'.

This is the type of lead employed by Craib (1928) for his records *c* and *d*, plate 1, which have the form of our figure *c*, plate 1. Such a record may be analyzed into two diphasic effects, one (fig. 5, *a*) due to the active depolarization of the membrane under each electrode in succession, the other (fig. 5, *b*) due to the leads which are, in effect, from the oppositely changing potential gradients in the core. To the latter, the membrane acts as a battery interposed between core and electrode, that under one electrode being charged to a potential difference equal and opposite to that under the other. Thus their potentials are without effect on current through the galvanometer until, with activity, one or the other potential is abolished.

It seems apparent to the authors that the possibility of a "triphasic" record is to be anticipated on the basis of the core conductor theory, although the initial positivity recorded ahead of the excitation proper is not a general condition characteristic of the muscle surface, but one locally produced by the arrangement of the circuit. Due to asymmetry of the external circuits on opposite aspects of the muscle, the muscle membranes are depolarized to different degrees at different points, depending upon different resistances in the external circuits which are in series with such regions of the membrane. The core current beneath a relatively insulated region of the membrane is chiefly the current which finds its outlet through less effectively insulated regions. Any two similarly insulated and unexcited surfaces, since neither is actively depolarized, have equal differences of potential from the core. The difference of potential between them therefore is effectively the potential difference between two regions of the core. To infer from such a situation that an electrode leading from the solution about the muscle would show a similar positivity, or that the immersed muscle surface *in contact with the bath* as a whole does so, is not only gratuitous but highly improper (Craib, 1928, p. 59), and no figures are published by Craib for a simple muscle such as the sartorius to indicate that such a potential was observed. On his theory of dipoles, it seems to us that even in the bath near the muscle, triphasic potential changes should be in evidence.

An analogous argument applies to the potentials to be expected when one electrode is applied to the surface in question, the other to the medium surrounding the muscle.

It appears that the triphasic effect discussed in section 1 *supra* may be augmented by the effect discussed here, the two being in the same direction with reference to the potential of resting muscle. On closer analysis, however, they are seen to be two aspects of the same phenomenon, the flow of current and a consequent potential difference between two regions of a muscle surface which is active at a point. The former is a manifestation of the effect in the external circuit of the muscle, the latter in the internal

circuit. While the potential gradients in these circuits are in opposite directions, the effective leads are at opposite electrodes, the potential differences between the two electrodes therefore changing in the same direction due to each effect.

When one whole aspect of the muscle is exposed to air, outside the bath (Craib's (1928) record *o*) essentially the same conditions obtain, except that now the whole air surface is (relatively) insulated as the immediate electrode areas were before. It is no longer necessary to use tubular electrodes (figs. *a–d*, plate 1 obtained with yarn leads). Owing to the high resistance of the thin layer of conductor on this surface, the muscle membranes underlying it are not appreciably depolarized by flow of current toward the active region except immediately ahead of the excitation itself. On the other hand, the underlying potential gradients in the cores, determined by current flowing through the low resistance bath *below* the muscle, will extend far ahead of the excitation. The air surface along the as yet inactivated muscle will tend to be at a given potential difference from the core, that difference characteristic of the membrane polarization of resting muscle. It may thus be more positive nearer the excited point than further away, because the core is more positive there, due to currents flowing out through the bath in contact with the *opposite* surface of the muscle. The occasion for the appearance of this positivity, however, is not the low resistance of the bath underlying the muscle alone, but the *difference* between the conductivities of the external circuits on opposite aspects of the active fibers. Again the result is imposed by the peculiar conditions of the experiment; it follows directly from the core conductor assumptions applied to these conditions; it does not justify any generalization contrary to these assumptions based upon the supposition that the whole of such a muscle is equally affected by the partial immersion; and specifically, it does not support Craib's hypothesis, which demands a similar potential gradient in the bath itself. In our records the inactive fibers apparently act with respect to the active ones like the bath in contact with one aspect of the whole muscle, and the effect here discussed presumably explains those records in terms of the core conductor theory, one "diffuse" lead and one opposite "localized" one (section 1) giving the triphasic record.

If this analysis is valid for action potentials recorded from muscle, it should also apply to demarcation potentials led off from an injured muscle in contact with a conducting medium. One should then expect that a point near the injured region on the air surface of a partially submerged muscle, would be positive to a point further removed from this injury. This Craib finds to be the case. On the other hand, a point in the immersing medium near the injured region should be negative to any other point, in the medium, but further away, along the uninjured muscle. This might

be inferred to be the case from Craib's measurements (1928, fig. 4) but the fields immediately adjacent to the muscle have not been charted.

4. *The form of the potential variation at the excited point of skeletal muscle.* These experiments suggest an alternative to the technique we have previously employed for studying the form of monophasic muscle potentials. If the container is sufficiently large, and the distant electrode is distributed about the periphery of the bath symmetrically with respect to the muscle, the passage of the impulse along the muscle will tend to give a symmetrical potential record. A tin bucket employed for a bath is connected to ground, serving at the same time as an electrode and as an electrostatic shield to the preparation. The other (adjacent) electrode is placed at the middle of the muscle-air surface, the lower surface only being in contact with the bath. The muscle is mounted with that surface downward which in the body was next the skin, as this undissected surface offers a more uniform contact for the diffuse lead. We did not curarize, since curare has a complex depressant action upon muscle. The muscle is stimulated by electrodes placed upon its air surface, at the uninjured pelvic end, and over fibers that conduct throughout the length of the muscle, as indicated by uniformity of the negative sign from an exploring electrode moved along the conducting pathway. The diphasic record appears as figure *C* 3, being the algebraic sum of the potentials recorded simultaneously at the two effective leads, both electrodes changing in potential with respect to the inactive muscle.

Under these circumstances, potentials similar to Craib's "triphasic" ones invariably show the third phase less conspicuous than the first. This we take to be due to the form of the falling phase of the potential of excitation. The fling of the galvanometer string is perhaps about sufficient to obscure this effect in Craib's records, and make the triplets appear symmetrical about the crest of the main deflection.

The asymmetry of the potential form indicated consists of a prolongation of the falling phase of the monophasic potential which we have discussed previously. The interpretation offered (Bishop and Gilson, 1927) is that the curve shows two superimposed effects, the potential signs of the two different types of activity in muscle, excitation or propagation of the impulse, and contraction. As has previously been noted concerning nerve (Bishop, Erlanger and Gasser, 1926) a strictly monophasic record is probably an impossible one to obtain directly; the technique might, by further analysis, result in another approximate derivation of the potential form.

Discussion. The question of the character of the potential changes in active muscle seems to us to merit the emphasis we have placed upon it in this paper, because no work from this laboratory in recent years has

been inconsistent with the Bernstein and Lillie hypotheses, allowing for such minor modifications as more accurate data seem to warrant. Before it devolves upon us to reinterpret nerve and muscle function in a fundamentally different manner, we think it appropriate to apply the assumptions already found to be so adequate, to such complications of the usual tissue potential record as may appear to be capable of a novel interpretation.

We believe the analysis presented herewith will also apply to other tissues, such as nerve, where the usual situation is simpler, and to heart muscle, where it is certainly more complex. An adequate application of the core conductor theory to complex tissues would demand the consideration of all structures which affect current flow. These may be active physiologically (i.e., electrochemically) or act only as physical reactance; and their anatomical (i.e., spatial) distribution involves Kirchoff's laws of a divided circuit as a complication of the law of Ohm. These considerations are being applied to heart tissue specifically by one of us (A. S. G.) and to nerve by the other. Until this work is completed we are unable to amplify the analysis to include effects which the reactance of the physiologically inactive structures must have upon tissue currents, or to discuss more particularly the internal structure of a cell so complicated as a muscle fiber.

As Craib infers, any potentials observed are those not at the source of current, but at some distance in the environment. Further complication of the environment naturally complicates further the recorded potentials, rendering them still less significant of the actual events at the tissue potential source.

In a simple symmetrical tissue the potential record seems to us to be a more direct, though still not an exact measure of the event underlying it when the environment is also simple. For this reason it has been the practice in this laboratory to removè all extraneous matter possible, such as the connective tissue sheaths of nerves, recognizing such adventitious material as the source of numerous artefactual alterations of an essentially simple potential form. All of these alterations, we have interpreted so far on the basis of the core conductor theory (Bishop, Erlanger and Gasser, 1926). Such simplicity even as nerve exhibits is not to be attained so easily in all tissues. The asymmetry of the environment with the tissue *in situ* (electrocardiogram) can, of course, be imitated by an asymmetrical bath; the asymmetry of the heart itself, however, is not compensated even by a symmetrical bath, but the character of the path of the impulse can perhaps be inferred from the potential record. We see no complications so far in applying the conventional theory to all electrically active tissues.

SUMMARY

Potentials are recorded from the frog sartorius muscle by means of the oscillograph, operated at high sensitivity, only a few fibers being stimulated. The records show a form which varies with the position of the electrodes on the muscle surface with respect to the active fibers.

When the electrodes are exactly upon active fibers, the process conducted past each electrode appears to be "triphasic;" when off this pathway each process is "monophasic."

Each triphasic record can be interpreted as the algebraic sum of single "phases" in opposite directions. One is due to the active depolarization process under the electrode, the other to the currents flowing from an active region through the cores of the fibers, and returning through inactive fibers which shunt the few active ones.

The results indicate that the conditions for obtaining such "triphasic" records are that an active fiber be *asymmetrically* shunted. The triphasic form is due to the greater duration of one phenomenon at the electrode region, the other reversing this in its middle course. The beginning and end of the first process thus appear as the two "positive" phases.

The form of this potential is consistent with the core conductor-membrane theory of muscle and nerve, and follows as a direct consequence of its fundamental assumptions. It is also consistent with the interpretation of the character of the electrical phenomena in muscle arrived at by the authors in a previous research.

The interpretation here proposed appears to be adequate to explain the electrical records of the heart, and is consistent with all variants of the nerve record as shown by the oscillograph.

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THE EFFECT OF REPEATED INJECTIONS OF EPINEPHRIN ON THE RESISTANCE OF SUPRARENALECTOMIZED RATS TO HISTAMIN POISONING

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The increased susceptibility of suprarenalectomized animals to histamin and other poisons has been well established (1–12). In a previous communication one of us (7) demonstrated that the height of the susceptibility of suprarenalectomized rats is reached at the end of the first week after the operation. Of 31 rats injected with 100 mgm. per kgm. of histamin (ergamine acid phosphate) on the fifth or seventh day following the removal of the suprarenal glands, 24 died. In an additional series of 14 suprarenalectomized rats injected with 100 mgm. of histamin per kgm. on the seventh day following the operation, 13 died.

In an effort to determine the rôle of medullary insufficiency in this increased susceptibility, the effect of injections of epinephrin on the protective mechanism of suprarenalectomized rats to histamin poisoning was studied. The experiments were divided into three groups.

In the first group 39 adult albino rats were suprarenalectomized and injected subcutaneously twice daily with epinephrin in amounts of 0.2 mgm. per kgm. from the day of operation to the day of histamin injection. The last injection of epinephrin was given two hours prior to the injection of histamin on the seventh day following the operation. The epinephrin used in all experiments was Mulford's "Adrin" (1/1000). This was diluted with physiological salt solution to make a 1/10000 dilution. One cubic centimeter therefore contained about 0.1 mgm. of epinephrin. The total quantity of fluid in each injection was brought up to 0.5 cc. with physiological salt solution. Six uninjected suprarenalectomized rats and 6 suprarenalectomized rats injected with the same quantities of physiological salt solution were included in this series as controls.

Of the 39 suprarenalectomized rats treated with subcutaneous injections of epinephrin up to and including the day of injection of the histamin, 19 or 49 per cent survived the injection of 100 mgm. of histamin per kgm. About half the rats, therefore, were protected against a lethal dose of histamin. Of the 6 control suprarenalectomized rats injected subcutaneously with physiological saline, only one survived the injection of histamin. Of

those suprarenalectomized rats receiving no injections, all died. This result indicated a definite protective action of epinephrin in these rats.

A second group of experiments was carried out to determine whether the protective action of epinephrin was dependent on the final injection and its possible pharmacologically antagonistic action to histamin. Twenty-five rats were suprarenalectomized. These received a single subcutaneous injection of epinephrin of 0.2 mgm. per kgm. two hours prior to the injection of histamin on the seventh day following the operation. Only 2 or 8 per cent of these rats survived. It would appear, therefore, that the protective action of epinephrin is dependent not so much on its immediate antagonistic action as on its continuous effect on a protective systemic mechanism.

In a third group the epinephrin was injected during six days following suprarenalectomy but the final injection was administered twenty-four hours prior to the injection of histamine. Of 13 rats treated in this manner 3 or 23 per cent survived.

These three groups of experiments indicate that the subcutaneous injection of epinephrin administered in the quantities we used during the entire period following the operation afford protection to suprarenalectomized rats against the lethal doses of histamin in a large percentage of instances. The last injection of epinephrin, however, must be administered on the day the histamin is injected. If the epinephrin is discontinued twenty-four hours prior to the injection of the histamin its protective effect is markedly decreased. On the other hand, a single injection of epinephrin given two hours prior to the injection of the histamin affords practically no protection.

Discussion. Epinephrin injected subcutaneously is absorbed into the blood stream at a constant rate over a long period of time. This has been adequately demonstrated by the work of Auer and Meltzer (13), Tatum (14), and Cori and Cori (15). Cannon and Rapport (16) present evidence to show that in cats approximately 0.0007 mgm. per kgm. per minute of epinephrin is secreted into the blood stream from the suprarenal glands. In cats in which the suprarenals have been removed this quantity of epinephrin injected intravenously is necessary to restore the pulse to normal, and they believe this amount, therefore, approximates the constant rate of secretion of epinephrin from the glands into the blood stream. Cori and Cori estimated the approximate rate of absorption from subcutaneous injections of epinephrin in amounts of 0.2 mgm. per kgm. They reasoned that, since the effect of subcutaneous injections of epinephrin on the blood sugar concentrations persists for at least four hours and since the effect per hour is constant and therefore the rate of absorption is also constant, the rate of absorption of epinephrin may be calculated as 0.2/240 or 0.0008 mgm. per kgm. per minute. This, it will be noted, is approximately the same as the physiological rate of secretion from the suprarenal glands of

cats as estimated by Cannon and Rapport. It may be assumed, therefore, that subcutaneous injections of epinephrin as administered in our experiments yield a constant supply of epinephrin to the blood stream over a long period of time.

The effects of histamin may be counteracted by injections of epinephrin both in normal and suprarenalectomized animals. The results obtained in our experiments, however, cannot be interpreted as due entirely to a direct pharmacologically antagonistic action of epinephrin to histamin. Such an explanation might be possible when the epinephrin is injected at the same time, immediately before or immediately after the injection of the histamin. Since these experiments were undertaken, Wyman (17) reports that he was able to protect suprarenalectomized rats against histamin poisoning by intraperitoneal injections of epinephrin. As indicated in his data, 34 rats were treated with epinephrin. One group of 12 rats received three intraperitoneal injections of epinephrin prior to the injection of histamin. Of this group 9 rats or 75 per cent survived. However, of 22 rats in a second group receiving only a single intraperitoneal injection of epinephrin ten minutes prior to the histamin, 3 or 13 per cent survived. This is about the percentage of survival of untreated suprarenalectomized rats injected with the same quantity of histamin. It is Wyman's contention that the small amount of epinephrin present in the blood stream following its injection intraperitoneally is responsible for its protective action against the histamin and is dependent on the immediate antagonistic effect of these two drugs. Since a single intraperitoneal injection of epinephrin administered ten minutes prior to the histamin failed to protect, this interpretation is unsupported by the data given. Apparently protection was not obtained by a direct antagonistic action of injected epinephrin co-incidentally circulating in the blood stream. Wyman's work is open to criticism in that he failed to keep the factor of time interval between operation and injection of histamin constant. The interval between operation and injection was more than three weeks in all his rats and in many, five to eight weeks. It has been repeatedly demonstrated in this laboratory and by other workers that the height of susceptibility of suprarenalectomized rats to histamin and other poisons occurs at the end of the first week following the operation and that subsequently rats become increasingly more resistant to these poisons though they never react entirely as normal rats. There is a compensatory hypertrophy of accessory cortical tissue and a hyperplasia of the lymphatic system and thymus gland two weeks after suprarenalectomy in a large percentage of rats (18). Therefore, the degree of susceptibility after this time is not as uniform as in the first week after suprarenalectomy. Moreover, other experimental conditions in Wyman's work were not kept constant. The dilution of epinephrin, the quantity

of epinephrin injected, the number of injections given and even the dosage of the histamin were all varied many times in his series of 34 rats.

If the action of epinephrin recorded in Wyman's paper were purely an antagonistic one to histamin, we should have to assume from this that the epinephrin repeatedly injected during a period of several days has a cumulative effect on the organism. This is contrary to the present day conception of the pharmacology of epinephrin. It is well known that epinephrin disappears rapidly from the blood stream. It is possible that repeated injections of epinephrin maintain the nutrition or tone of some systemic mechanism (autonomic nervous system?) and increase the resistance of suprarenalectomized rats to histamin poisoning in a manner not accounted for on a purely pharmacologically antagonistic basis.

Is the susceptibility of suprarenalectomized rats to histamin poisoning to be considered then a manifestation of medullary or cortical insufficiency or both? Wyman (17) found that transplants of small fragments of cortical tissue are insufficient to protect suprarenalectomized rats against histamin. From this he concludes that susceptibility to histamin poisoning in these animals is due to insufficiency of the medulla and not of the cortex of the suprarenal gland. However, it has been our experience that even a small fragment of cortical tissue left in situ is sufficient to protect suprarenalectomized rats against injections of lethal doses of histamin. Kellaway and Cowell (9) have shown that if the cortex of both suprarenal glands of cats are destroyed but the medulla of one gland left in situ, the animals show a marked hypersensitivity to small doses of histamin. They believe that deficiency of the cortex plays an important part in the susceptibility of suprarenalectomized animals to histamin. Certainly the evidence obtained from studies on histamin poisoning does not help in differentiating cortical from chromaffin function.

SUMMARY

1. Subcutaneous injections of epinephrin administered twice daily during seven days following suprarenalectomy afford protection to rats against lethal doses of histamin in 50 per cent of cases. The last injection of epinephrin was administered about two hours prior to the injection of histamin.

2. If the epinephrin is discontinued twenty-four hours before the injection of histamin its protective effect is markedly decreased.

3. A single injection of epinephrin given two hours prior to the injection of histamin affords no protection to suprarenalectomized rats.

We believe that the protective action of repeated subcutaneous injections of epinephrin in suprarenalectomized rats against histamin poisoning is in part due to a true hormonal effect and is not entirely dependent on the pharmacological antagonistic action of epinephrin to histamin.

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PHYSIOLOGICAL VARIATIONS IN THE CARDIAC OUTPUT OF MAN[1]

II. Changes in the Cardiac Output, Metabolism, Blood Pressure, and Pulse Rate of Man Following the Ingestion of Fluids

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The effect of ingesting fluids on the cardiac output of man has been determined heretofore only by Jarish and Liljestrand (1927). These investigators found that the administration of 1.3 liters of water or sugar solution resulted in an increase usually of about 50 per cent or more in the cardiac output of the two subjects whom they studied. Their paper gives one the impression that this great increase in cardiac output is the normal resultant of fluid ingestion although they state that the result could not always be duplicated on one of their two subjects. A knowledge of the changes in cardiac output resulting from fluid ingestion is of importance for such light as it might throw on the problem of the regulation of the heart. The problem has also certain clinical interest because of the widespread practice of forcing fluids.

Methods. The determinations of the cardiac output were made by the use of acetylene as described in a previous communication (Grollman, 1929). This gas has continued to give the satisfactory results described in its original presentation.[2] The analyses were carried out in an apparatus equipped with an 11 cc. pipette and are considered as accurate within 5 per cent. The subjects used in the present study were all young, healthy adults in the third decade of life. The experiments were usually performed in the morning as soon as the subjects arrived in the laboratory. They had taken no fluids or food since the previous evening and were thus in the

[1] Paper I of this series (Grollman, 1928) considered the variation of cardiac output with varying postures.

[2] When high concentrations (20 per cent) of acetylene are rebreathed, a transient and mild form of anesthesia may result. With still higher concentrations of acetylene (40 per cent) in the rebreathing mixture, tingling of the extremities may result. Both of these effects are of momentary duration and leave no after effects. In the method advocated for the determination of the cardiac output there is never any occasion for using acetylene in a concentration greater than 10 per cent, under which conditions none of the above mentioned effects are encountered.

basal condition. After at least one-half hour of rest reclining in a steamer-chair, the experiment was commenced. After determining the pulse rate, oxygen consumption, and blood flow in the basal condition, the subject drank from 1000 to 1500 cc. of water or 1 per cent NaCl or Locke's solution, and the determinations were repeated, at intervals, for several hours afterwards. In certain cases, blood pressure readings were also taken by the auscultatory method. The rate of diuresis was also recorded. In the early experiments the ingested fluid was of room temperature, but in order to avoid any possible effects of cooling on the cardiac output, the ingested fluids were, in the later experiments, warmed to body temperature (38°C.).

PROTOCOL 1

Subject N. C. ♂. Height, 185 cm.; weight 81 kgm. Basal. Resting from 8:50 a.m. Drank 1200 cc. water, at 38°, from 9:40 to 9:43 a.m.

TIME	PULSE	URINARY EXCRETION	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
	rate per minute	*cc. per minute*	*cc. per minute*	*cc. per liter*	*liters per minute*
9:25		1.0	253		
9:35	70			61	4.15
9:50			266		
10:00	64	1.0		51	5.21
10:22			262		
10:32				52	5.04
10:52	64	10.2	260		
11:05				54	4.81
11:30	60		256		
11:39		12.2		57	4.49
11:47	60		253		
12:00		10.0		61	4.15
12:30	60		252		
12:40		2.5		60	4.20

In order to avoid any variability in the observed results due to changes in the room temperature, the latter was maintained approximately constant at 22°C. except in the experiment cited as protocol 3, where there was a gradual rise in the temperature of the room from 18° to 23° during the course of the experiment.

Results. Results of typical experiments are given in the accompanying protocols. These protocols were selected of all the experiments performed, because they illustrate the type of response which was found to follow the ingestion of fluids. Other experiments, not recorded here, showed results similar to the protocols cited.

The only significant conclusion that could be adduced from the changes in pulse rate occurring after the ingestion of fluid, is that there is no

apparent relation between the pulse rate and the cardiac output or other factors which were determined. These results indicate, however, a marked variation of the stroke-volume for an individual during a given experiment. The blood pressure measurements were too few in number to permit of generalizations but in general, as reference to protocols 2 and 5 indicates, the blood pressure changes are reflected in the cardiac output. They show, on the other hand, no relation to the rate of urinary excretion.

The effect of the ingestion of fluids on the respiratory metabolism. After the ingestion of water, a rise in the respiratory metabolism was always encountered. In most cases the maximum rise was about 5 per cent (protocol 1) but in many cases it amounted to 10 per cent (protocol 2) and

PROTOCOL 2

Subject A. C. ♀. Basal. Height, 158 cm.; weight 55 kgm. Basal. Resting from 7: 40 a.m. Between 8: 40 and 8: 45 the subject drank 1000 cc. of water at 38°

TIME	PULSE	BLOOD PRESSURE	RATE OF URINARY EXCRETION	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
		mm. Hg	*cc. per minute*	*cc. per minute*	*cc. per liter*	*liters per minute*
8:10	66					
8:20	66	99/75	0.8	183		
8:35					60	3.05
8:45				200		
9:00		113/70			70	2.86
9:15	56	106/68	1.0	197		
9:25	62				58	3.41
9:35			10.0	196		
9:45	60	108/74			60	3.27
9:55			13.4	184		
10:10	60				62	3.00
10:30		100/75	8.0	182		
10:40	60				60	3.03

occasionally to more. The rise in the respiratory metabolism following the ingestion of water has been recently studied by Lublin (1928) who considers it to represent the extra energy required by the kidneys to excrete the water ingested. Despite the work carried out on renal metabolism (Barcroft, 1908; Zuntz, 1910; Tangl, 1911, 1913; Janssen and Rein, 1927) uncertainty still exists as to the ultimate cause of this metabolic rise. The increase in protein metabolism which has been observed by a number of workers to follow the ingestion of water (Lusk, 1928), although attributed by some to a removal of accumulated nitrogenous waste products, is no doubt, in part at least, a concomitant of the increased respiratory metabolism. The results of the present investigation show a very rapid rise in the oxygen consumption soon after the ingestion of water and a gradual drop in the

metabolism with time. This metabolic change, however, does not run parallel to the rate of diuresis which reaches its maximum when in many cases the respiratory metabolism has diminished almost to normal. It would appear, therefore, that the diuresis and metabolic rise are not related and that the latter is not caused by an increase in the energy utilized by renal activity but results from a general stimulation of the metabolism of the cells of the body. This stimulation can be considered as due to the dilution of the internal environment of the cells by the ingested fluid. When the diluting fluid has the same concentration as the cell environment, e.g., after ingestion of isotonic saline or Locke's solution, no change occurs in the respiratory metabolism (protocols 4 and 5).

PROTOCOL 3

Subject A. C. ♀. Basal. Height, 158 cm.; weight 55 kgm. Resting from 7:30 a.m. Drank 1000 cc. of water at 15°C. from 8:25 to 8:30 a.m. The subject experienced a chill at 8:40 and felt subjectively chilly until about 9:30 a.m.

TIME	PULSE	RATE OF URINARY EXCRETION	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
		cc. per minute	*cc. per minute*	*cc. per liter*	*liters per minute*
8:20	60		180		
8:25		0.5		60	3.00
8:40	55		185		
8:50	54			98	1.89
9:00		5.5	203		
9:10	55			90	2.24
9:20		11.2	200		
9:30	55	12.8		86	2.32
9:50		12.8	185		
10:00	56	12.0		70	2.67
10:30			180		
10:40		5.0		60	3.00

Changes in the cardiac output following the ingestion of water, isotonic saline or Locke's solution. Protocol 1 shows the rise in the cardiac output which sometimes follows the ingestion of a large quantity of water. The heart output in this case rose immediately after the ingestion of the fluid to 26 per cent above its basal value and then gradually declined again to its original value. This rise in the cardiac output was the maximum ever observed during the course of this study. Jarisch and Liljestrand (1927) report changes in the cardiac output which were often almost 100 per cent higher than the basal values, but no such changes were ever encountered in this study. These investigators record only isolated determinations and the method which they employed is subject to gross errors which possibly vitiate their results.

The comparatively large increase in cardiac output shown in protocol 1 was not frequently observed. In fact, the commonest type of response is shown in protocol 2 where the maximum rise is 12 per cent above the basal value. In some cases no increase occurred, as was also observed by Jarisch and Liljestrand (1927). A possible explanation of the difference in response in different experiments may be found perhaps in the different states of hydration in which the subjects were at the beginning of the experiment. If, as shall be discussed later, the increased cardiac output is of reflex nervous rather than direct chemical origin, individual variations would be naturally expected.

Immediately after the ingestion of the water, the cardiac output of the subject in protocol 2 decreased. This decrease was observed on several

PROTOCOL 4

Subject H. G. ♂. Height, 178 cm., weight 68 kgm. Basal. Resting from 9:00 a.m. Drank 1300 cc. of isotonic saline solution, at 38°C., from 9:40 to 9:53 a.m.

TIME	PULSE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
	rate per minute	*cc. per minute*	*cc. per liter*	*liters per minute*
9:30	60	220		
9:40			60	3.67
10:00	66	220		
10:13			40	5.50
10:26	66	222		
10:40			40	5.55
10:53	66	218		
11:07			47	4.64
11:30	66	220		
11:42			60	3.67

individuals and possibly denotes a reflex effect resulting from the sudden distention of the stomach by the mass of water administered. In the case of protocol 3, in which the ingested water was cold, the subject experienced a chill and remained subjectively chilly for several hours. The heart output in this case decreased over 30 per cent and gradually rose to its original value after 2 hours. This result was not obtained on other subjects and is possibly related to the effects of the low temperature of the water and its subsequent chilling effect.

The rise in the cardiac output was much more general after the ingestion of isotonic saline or Locke's solution than after the ingestion of pure water. Protocols 4 and 5 show the extreme type of results obtained. The great rise shown in protocol 4 did not occur frequently. The smaller rise shown in protocol 5 was the usual reaction to the ingestion of saline. In every case studied there was some rise (usually about 8 to 12 per cent) in the cardiac output.

The observed rise in cardiac output is an accompaniment of the increased oxygen consumption, the decreased arterio-venous oxygen difference, or both of these factors. As regards its causation, several possibilities occur. The fact that the increase in cardiac output does not run *pari passu* with the urinary excretion would indicate that, like the pulse and blood pressure, it is not related to the increased renal activity. Moreover the cardiac output is increased to a greater extent after the ingestion of isotonic saline or Locke's solution, where no diuresis occurs. We might attribute the rise in cardiac output to the dilution of the blood which accompanies water ingestion, but the latter is so small[3] (Haldane and Priestley, 1915–16; Siebeck, 1918; Priestley, 1921) that this explanation seems rather improb-

PROTOCOL 5

Subject A. G. ♂. Height, 164 cm.; weight, 63 kgm. Basal. Resting from 7:30 a.m. Drank 1350 cc. of Locke's solution, at 25°, from 8:13 to 8:23

TIME	PULSE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
		mm. Hg	*cc. per minute*	*cc. per liter*	*liters per minute*
8:00	64	90/68	230		
8:10	64			60	3.83
8:30			230		
8:40	62	108/80		60	3.83
8:50			230		
9:00	66			57	4.04
9:10	66	100/75	230		
9:25				57	4.04
9:35			230		
9:50	67	94/70		60	3.83

able. A more pronounced change occurs in the electrical conductivity of the blood (Priestley, 1915–16; Rioch, 1927) and the changes in cardiac output, indeed, seem to parallel the conductivity changes as regards their time of occurrence. If the electrical conductivity, that is, the concentration of ionizable electrolytes, regulated the cardiac output, one would expect a decrease after the ingestion of Locke's solution, where the conductivity is increased. A more general increase, as noted above, was, however, obtained under these conditions. It seems most probable, therefore, to consider the changes in cardiac output which follow the ingestion of fluids, as resulting from reflex changes brought about by other than chemical agencies. The most obvious such factor is the production of a state of plethora in the vascular system. The fact that the blood is only slightly diluted after the ingestion of fluids is no indication of the degree of plethora

[3] Marx (1926) describes rather marked dilution of the blood as occurring after the ingestion of water. His results, however, are in contradiction of those of other observers.

produced. There would be a tendency for any dilution of the salts, proteins or hemoglobin of the blood to be compensated for by the passage of salts and proteins from the tissues and of erythrocytes from the spleen or other reservoirs. Assuming such an equilibrium to be established between the blood and tissues one would expect a passage of most of the ingested water (following water ingestion) into the tissues while in the case of the ingestion of isotonic saline or Locke's solution, more of the ingested fluid would tend to remain in the vascular system.[4] The greater increase in cardiac output following ingestion of Locke's solution would be due, therefore, to the greater condition of plethora which this produces in the vascular system.

SUMMARY

A study was made of the pulse rate, blood pressure, rate of urinary excretion, oxygen consumption, arterio-venous oxygen difference, and cardiac output of man after the ingestion of 1000 to 1500 cc. of water, 1 per cent NaCl solution, or Locke's solution. The cardiac output after the ingestion of water may rise as high as 26 per cent, above its basal value, but usually there is only a slight increase (about 10 per cent) and in some cases no change occurs. After the ingestion of saline the rise is more marked and though usually only about 10 per cent it may be as much as 50 per cent above the basal value. The respiratory metabolism is increased, on an average, 8 per cent by the ingestion of large quantities of water. Locke's or 1 per cent NaCl solutions produce no perceptible effect. These changes and their relation to the pulse rate, blood pressure, and rate of diuresis after fluid ingestion, are discussed.

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[4] This difference in behaviour towards water and isotonic solutions results from the ability of the tissues to maintain a constant osmotic pressure within their constituent cells.

THE HYDROGEN ION CONCENTRATION OF THE REPRODUCTIVE ORGANS OF THE WHITE LEGHORN CHICKEN[1]

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From the Kentucky Agricultural Experiment Station, Lexington

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In reviewing the literature on the physiological and chemical causes of sterility we were unable to find any reference to the hydrogen ion concentration of the reproductive organs and their secretions, of the white Leghorn chicken and but few references concerning this reaction in the human and in animals.

Randall and Muschat (1926) have observed pH values from 7.4 to 7.6, with an average of 7.5, for human semen. They have also shown that spermatozoa are inactive in a medium having greater acidity than pH 6.0 and that activity increases with increasing alkalinity until the maximum is reached between pH 8.5 and pH 9.5, and as the alkalinity increases above this, the activity decreases. Their experiments have shown that human spermatozoa may remain immotile in a medium having a pH value of 4.0 for 30 minutes and retain the power to become motile when the medium is made alkaline.

Behrens and Narejak (1925) have shown that the vaginal secretion of the normal female with the vaginal bacilli predominating has a pH value ranging from 3.85 to 4.45, that when there is an admixture of cocci the pH is between 4.6 and 5.34 and when a large number of cocci and particularly streptococci are present, the pH lies between 4.9 and 6.04.

Gragert (1925) has shown that in the new-born female child, the sterile vaginal secretions are neutral. Following bacterial invasion, these secretions soon become acid and after a few days show a pH of 6.7 which he states is normal for the healthy adult female and that it does not change during pregnancy.

Pasch (1922) has made observations showing that, unlike the conditions existing in woman, the vaginal secretions of the guinea pig, rabbit and cow are alkaline.

Anderson, Peter and Healy (1921) have observed pH values ranging

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Mr. E. S. Hill assisted in making some of the observations reported in this paper.

from 6.94 to 7.51, with an average of 7.31, for normal horse semen and pH values from 7.49 to 7.76, with an average of 7.58, for fresh horse semen in which the spermatozoa were not motile.

In considering the biology of fertility of the male chicken, practical experience has shown that the ability to fertilize ova is at its height the first season and remains high through the next two breeding seasons, after which it declines and cocks are usually removed from breeding flocks and no longer used for this purpose.

Practical experience also teaches that fertilized eggs should not be kept longer than 14 days before incubation in order to insure good hatches.

Unpublished experimental data obtained by W. S. Anderson and J. Holmes Martin of this Station show that where white Leghorn males mate as often as 25 or 30 times a day, about two-thirds of the matings do not result in fertilization. This is due to the inability of the male to furnish all the females sufficient seminal material to cause fertilization. It was found that a vigorous white Leghorn male, after having been separated from females for several days, would mate every three or four minutes if virgin females were placed with him. The male does not space his sexual activity so that he can accumulate the quantity of semen necessary to make impregnation possible.

Sexual excess in man or animal results in depletion of seminal material and temporary infertility is a consequence. The sperm cells must be matured by a vital process. Their growth and maturity cannot be hastened by sexual activity. It is true, however, that sexual activity may, and often does, increase the secretion of the secondary sex glands, which dilutes the secretions of the reprodutive glands; but it must be remembered that the secretion ejaculated from the accessary sex glands is of no value for fertilization unless it contains a liberal number of sperm cells. Excessive sexual activity, all things being equal, reduces or exhausts the supply of sperm cells without which impregnation is impossible.

The experiment described herein is part of an extensive investigation to determine the chemical reactions occurring during the process of egg formation in the laying hen and particularly to determine the chemical changes involved in the transference of calcium from the ingested food material in the alimentary canal, by means of the blood stream, to the oviduct, and its deposition in the form of calcium carbonate as the egg shell.

Since the secretion of the several materials necessary to complete the formation of the egg after the ripe ovum has entered the oviduct is primarily a process of dialysis, our first problem was to determine the hydrogen ion concentration of these secretions and of the different tissues that secrete them.

The oviduct of the hen is divided into five sections having different functions. The funnel, or upper part of the oviduct, receives the fully

formed ovum or yolk when it is discharged from the ovary. The yolk then passes from the funnel into the albumin-secreting part where the largest part, if not all, of the albumin is placed around it. The thin wall of the funnel expands or merges into the thicker longitudinal ridges of the albumin-secreting section which is joined abruptly with the third division called the isthmus by a band, the structure of which makes a clear line of demarcation. The mucous membrane folds are lower and become gradually higher in the isthmus but never reach the height of those in the preceding section. The band lacks the long, tubular glands and the cores of the folds of the mucous membrane contain more connective tissue. Otherwise the histology of those two portions is the same. The function of the isthmus is to form the two egg shell membranes about the white of the egg.

TABLE 1

Lengths of the different parts of the oviduct of laying and non-laying white Leghorn hens

PART OF OVIDUCT	HEN THAT NEVER LAID	HEN NOT IN LAYING CONDITION	AVERAGE OF FIVE LAYING HENS
	cm.	*cm.*	*cm.*
Funnel	2	2	4
Albumin-secreting part	6	15	36
Membrane-secreting part	2	3	5
Shell-secreting part	3	5	7
Bloom-secreting part	5	7	10
Total	18	32	62

The longitudinal mucous folds of the isthmus are continuous and merge into the larger and deeper folds of the uterus which secrete the calcium carbonate for the egg shell.

The last part of the oviduct, which is generally designated as the vagina, is separated from the uterus by a strong sphincter muscle and has a different histological structure. Here the shell cuticle or bloom is secreted.

Five hens that were actively laying, one that never laid, one that was in a partial molt and had practically stopped laying and one that was in a complete molt and had not laid an egg for 20 days were selected from the flock of white Leghorns. These were killed by breaking their necks, their oviducts dissected out immediately and each divided into the 5 sections as mentioned above, which had the lengths given in table 1.

The separate parts were opened longitudinally and some egg albumin was removed from the mucous folds of each. Small portions were placed on white porcelain test plates and the hydrogen ion concentration determined colorimetrically, using dilute indicator solutions as recommended by Healy (1927).

The egg albumin from an egg that had been kept in a room having a temperature of 60°F. for 20 days, a fresh laid egg, a shell-less and membrane-less egg found in the isthmus of a hen, and a shell-less egg found in the uterus of a hen were tested in like manner. Our observations show that the hydrogen ion concentration of the egg albumin of a fresh laid egg is pH 8.2 and that of an egg that is 20 days old is approximately pH 9.8. This increased alkalinity is probably due to the loss of CO_2 and auto-digestion of the proteins in a sterile egg. In a non-sterile egg the liberation of ammonia due to bacteria may also play a part.

After the secretions had been carefully removed from the mucous membranes lining the walls of the separate portions of the uterus by the aid of filter paper, a small part of the mucous membrane folds was cut off and placed on the testing plate, mashed thoroughly and the colorimetrical solutions added and stirred. The colors developed were compared with the Clark color chart.

The egg albumin from a shell-less egg found in the uterus was pH 7.4 and that from a shell-less and membrane-less egg found in the isthmus was pH 7.4. The egg albumin taken from the walls of the albumin-secreting mucosa gave pH values of 6.7 or 6.8 while that taken from the mucosa walls of the uterus was slightly alkaline having a range of pH from 7.3 to 7.5. The alkalinity of the egg albumin found in the uterus possibly is due to the calcium which is being brought to the uterus by the blood stream.

The hydrogen ion concentration of the mucosa of the funnel and of the upper and lower parts of the albumin-secreting section ranges between pH 6.3 and pH 6.6 in hens that are laying and not laying.

The hydrogen ion concentration of the mucosa of the isthmus, uterus and vagina of hens that are actively laying ranges between pH 5.6 and pH 5.9, while these same parts in hens that are not laying or nearly stopped laying are less acid, the pH values ranging from 6.2 to 6.6.

A white Leghorn cockerel and a yearling cock whose fertilizing abilities were known to be high were killed and the testicles and seminal vesicles immediately dissected out.

The semen removed from the vesicles, when examined microscopically, was found to contain many active spermatozoa. The hydrogen ion concentration was determined colorimetrically and in each case was found to be pH 7.3. This is approximately the same as that of the stallion and but slightly less alkaline than that of man.

In briefly discussing this experiment, the spermatozoa of the cock are deposited in the cloaca of the hen and must traverse a distance of 60 cm. through the oviduct before they can fertilize the ovum from the ovary. It is interesting to note that the spermatozoa existing normally in an alkaline secretion must travel the distance of 60 cm. through an organ that is acid in reaction.

Egg albumin as secreted in the oviduct is slightly acid in reaction and the tissue that secreted it is slightly acid. Shortly after an egg is laid, the albumin becomes increasingly more alkaline, possibly because of the loss of CO_2 and autodigestion of the protein. At the expiration of 20 days the pH value reaches 9.8 which confirms the observations of Healy and Peter (1925). It may be that this increasing alkalinity influences the hatchability of eggs.

TABLE 2

Hydrogen ion concentrations of egg albumin and the mucosa of the oviduct

	ACTIVELY LAYING HEN	ACTIVELY LAYING HEN	ACTIVELY LAYING HEN	ACTIVELY LAYING HEN	ACTIVELY LAYING HEN	HEN THAT NEVER LAID	HEN, NEARLY STOPPED LAYING*	HEN THAT HAD NOT LAID FOR 20 DAYS†
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
Egg albumin from shell-less egg in uterus					7.4			
Egg albumin from shell-less and membrane-less egg in isthmus	7.4							
Egg albumin from albumin-secreting mucosa	6.7	6.8	6.8	6.7	6.7			
Egg albumin from uterus mucosa	7.4	7.5	7.5	7.3	7.4			
Funnel mucosa	6.4	6.6	6.4	6.3	6.4	6.4	6.6	6.6
Upper albumin-secreting section mucosa	6.4	6.6	6.3	6.5	6.5	6.4	6.5	6.5
Lower albumin-secreting section mucosa	6.4	6.6	6.3	6.4	6.4	6.5	6.4	6.5
Isthmus mucosa	5.8	5.9	5.8	5.8	5.6	6.6	6.4	6.4
Uterus mucosa	5.8	5.9	5.8	5.8	5.9	6.2	6.3	6.4
Vagina mucosa	5.9	5.9	5.8	5.8	5.9	6.2	6.4	6.3

* Hen in partial molt.

† Hen in complete molt.

The hydrogen ion concentration of the mucosa of the oviduct in the hen that had never laid and in the two hens that had stopped laying ranged between pH 6.2 and pH 6.6, whereas in the actively laying hens the mucosa of the isthmus and uterus was distinctly more acid than that of the albumin-secreting part, the pH of the former ranging from 5.6 to 5.9, and of the latter, from 6.3 to 6.6. This suggests the possibility that this difference in acidity may be associated with the formation of the shell membrane and the egg shell.

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THE RELATIONSHIP BETWEEN CELL COUNT AND SEDIMENTATION INDEX

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Our previous work has shown a significant relationship between the fibrinogen content of plasma and the sedimentation rate (1, 2, 3). In order to gain further information regarding sedimentation, we have made a statistical study of the relationship between cell count and sedimentation index.

A brief review of the literature will be presented. Fahraeus (4) believed that the sedimentation velocity was increased by a decrease in the number of red cells. Gram (5) considered both fibrinogen content and cell volume important factors in sedimentation. Schumacher and Vogel (6) decided that more reliable information regarding inflammatory processes was derived from the sedimentation rate than from the leucocyte count. Baer and Reis (7) reached the same decision. One might interpret these conclusions as indicative of a lack of relationship between sedimentation velocity and leucocyte count.

Morriss (8) found no relationship between the rate of sedimentation and the red count, white count, or hemoglobin content. Rubin and Smith (9) believed that they had found a close relationship between the cell volume and the rate of sedimentation, but they denied such a relationship between the cell count and the sedimentation velocity. We have analyzed their published results and will state our findings in another section.

Schäfer (10) found that a faster rate of sedimentation accompanied a lower cell count. Vögel (11) found results which differed from those of Schäfer. Further reference will be made to his work. Hubbard and Geiger (12) found a distinct relationship between the rate of sedimentation and the number of red cells. Owing to the disagreement in the literature, we deemed it advisable to make a further study of the relationship between cell count and sedimentation velocity.

METHODS. The present study includes 50 men and 50 women. Of this number, 25 of each sex were students in attendance at the University of Minnesota, while the remaining 25 of each sex were patients with minor ailments being cared for in the out-patient department of the University Hospital. These two sources gave a fair range of cell count. There was no attempt to select patients of the same age, hence the extremes were 18 and

79 years. The red counts, white counts, and differential counts were all made by the same individual, in the usual manner. The sedimentation index was determined by the Cutler (14) method, using $\frac{1}{2}$ cc. of 4.4 per cent potassium oxalate instead of sodium citrate.

We prefer the Cutler method, with potassium oxalate, for the following reasons. The diameter of the tubes which we have used in all of our work is one centimeter. This width of tube allows the cells to fall freely, while a narrow one offers interference with the rate of fall of bodies of such size as the blood cells. Physical chemists, in personal communication, have criticized methods which make use of tubes of small diameter. This method requires a relatively large amount of blood ($4\frac{1}{2}$ cc.), so the same sample serves for fibrin determinations after the sedimentation test is completed. Potassium oxalate is chosen for the anticoagulant for two reasons. First, the fibrin determinations are readily carried out on oxalated plasma. Secondly, as shown by Rubin and Smith (9), the cell volume suffers less alteration in oxalate than in citrate. Consideration of the above factors has convinced us that this method is more desirable than any of the others available at present. The fibrin determinations were made in duplicate, by the Wu (15) colorimetric method, using the precautions mentioned in earlier papers.

Results. The mean number of red cells per cubic millimeter was 3,880,000 $\pm$35,000 for the 50 women, and 4,230,000 $\pm$61,000 for the 50 men. The difference between the sexes is 350,000 $\pm$70,000. The mean sedimentation index (one-hour reading) was 8.44 $\pm$0.44 mm. for the women and 5.68 $\pm$0.65 mm. for the men. The difference is 2.76 $\pm$0.78 mm. In order to determine the relationship between the number of cells and the rate of sedimentation, the usual statistical methods have been employed. This relationship is shown by a correlation coefficient, r_{RS}, of -0.319 ±0.086 for the women, and -0.662 ±0.054 for the men, where R = the number of red cells per cubic millimeter and S = the sedimentation index in millimeters. The difference between the correlation coefficients for the two sexes is 0.343 $\pm$0.101.

It seems appropriate to refer to Rubin and Smith's (9) results again at this time. Their cases included 22 men and 18 women. Of this number, 5 of each sex were individuals in good health, while the remainder were patients. Their data were published in the form of tables, from which they drew the conclusions stated in the introduction. We have analyzed their data with the results shown in table 1.

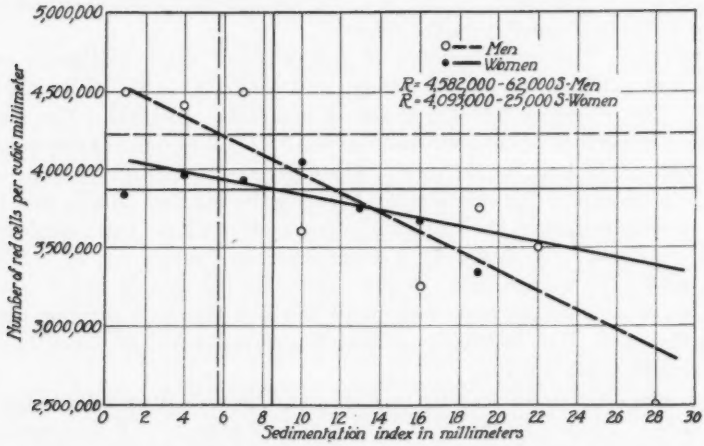
This analysis of the data shows their conclusions to be untenable. The relationship between the sedimentation rate and cell count, r_{RS}, is as significant in the men as is the relationship between the sedimentation rate and the cell volume, r_{VS}. The women show a lower degree of correlation in the two relationships, but both are significant. Since Rubin and

Smith's results are in such close agreement with our own, we are at a loss to understand those of Vögel (11). He found a coefficient, r_{RS}, of -0.01 for 48 healthy women, and one of -0.03 for 194 gynecological cases. The method used differed from ours, and may be responsible, in part, for the lack of agreement.

TABLE 1

	MEAN SEDIMENTATION IN 2 HOURS	MEAN CELL VOLUME	r_{VS}	MEAN CELL COUNT	r_{RS}
22 men.....	30.00 ±2.85 mm.	42.728 ±1.20	−0.841 ±0.042	4,272,800 ±135,000	−0.830 ±0.045
18 women.....	39.334 ±2.85 mm.	39.445 ±0.95	−0.775 ±0.063	3,777,800 ± 76,000	−0.477 ±0.120

Returning to our own results, we found the linear regression equation, calculated from the means, standard deviations, and correlation coefficient, to be $R = 4{,}093{,}000 - 25{,}000\ S$ for the women, and $R = 4{,}582{,}000 - 62{,}000\ S$ for the men, where, as above, R = the number of red cells per cubic millimeter, and S = the sedimentation index in millimeters. The actual means and the fitted regression line are shown in the graph.



The mean white count was 7,120 ±177 for the women and 7,800 ±187 for the men, with a difference of 680 ±257. The correlation coefficient, r_{WS}, is −0.020 ±0.095 for the women, and 0.070 ±0.095 for the men, where W = the number of white cells per cubic millimeter and S = the index in millimeters. The difference is 0.090 ±0.13. Thus, in this series, there is no significant relationship between the white count and the sedimentation rate in either sex.

The percentages of neutrophiles and lymphocytes were determined by

the differential count. The percentage of neutrophiles in the women was 67.20 ±0.91, and in the men it was 67.10 ±0.63, the difference being 0.10 ±1.10. The correlation coefficient, r_{NS}, is −0.092 ±0.095 for the women, and 0.122 ±0.094 for the men. The difference is 0.214 ±0.134. There is, in this series, no significant correlation between the percentage of neutrophiles and the sedimentation rate, in either sex.

A lymphocyte percentage of 28.80 ±0.88 was found for the women, and 27.50 ±0.52 for the men, the difference being 1.30 ±1.02. The correlation coefficient, r_{LS}, was 0.166 ±0.093 for the women, and −0.120 ±0.094 for the men, the difference being 0.286 ±0.132. The low values of these correlation coefficients, in comparison with their probable errors, and the

TABLE 2

	WOMEN	MEN	DIFFERENCES	DIFFERENCE / ERROR OF DIFFERENCE
Mean sedimentation index (50 cases)	8.44 ±0.44 mm.	5.68 ±0.65	2.76 ±0.78	3.5
Mean number red cells	3,880,000 ±35,000	4,230,000 ±61,000	350,000 ±70,000	5.0
Correlation coefficient, r_{RS}	−0.319 ±0.086	−0.662 ±0.054	0.343 ±0.101	3.4
Mean number white cells	7,120 ± 177	7,800 ± 187	680 ± 257	2.6
Correlation coefficient, r_{WS}	−0.020 ±0.095	0.070 ±0.095	0.090 ±0.13	0.7
Mean per cent neutrophiles	67.20 ±0.91	67.10 ±0.63	0.10 ±1.10	0.09
Correlation coefficient, r_{NS}	−0.092 ±0.095	0.122 ±0.094	0.214 ±0.134	1.6
Mean per cent lymphocytes	28.80 ±0.88	27.50 ±0.52	1.30 ±1.02	1.2
Correlation coefficient, r_{LS}	0.166 ±0.093	−0.120 ±0.094	0.286 ±0.132	2.1
Mean sedimentation index (49 cases)	8.35 ±0.45 mm.	5.78 ±0.66	2.57 ±0.80	3.2
Mean per cent fibrin	0.3245 ±0.0043	0.3194 ±0.0058	0.0051 ±0.0072	0.7
Correlation coefficient, r_{FS}	0.465 ±0.075	0.660 ±0.054	0.195 ±0.092	2.1

inconsistency in the signs of the coefficients for the two sexes indicate that no significant relationship exists between the percentage of lymphocytes and the rate of sedimentation in the present series.

In 49 cases of each sex the fibrin content was determined. The mean index for the 49 women was 8.35 ±0.45 mm., and for the 49 men it was 5.78 ±0.66 mm., with a sexual difference of 2.57 ±0.80 mm. The mean fibrin content was 0.3245 ±0.0043 gram per 100 cc. of plasma for the women, and 0.3194 ±0.0058 gram for the men, with a difference of 0.0051 ±0.0072 gram. That the sedimentation rate is significantly related to the fibrin content is shown by a correlation coefficient, r_{FS}, of 0.465 ±0.075 for the women, and 0.660 ±0.054 for the men. The difference is 0.195 ±0.092. These results are in accord with our previous work, consequently we shall not discuss them further.

We desire, however, to call attention to a paper by Pinner, Knowlton and Kelly (13). Their work includes the determinations of fibrin contents and sedimentation rates in 218 cases. A published chart presents the respective values found. They state that one glance at the chart shows that there is no "lawful relationship" between these two factors. However, examination of the chart caused us to doubt the absence of a "mathematical relationship;" consequently, we have made a statistical study of the data presented. The mean sedimentation index is 22.75 $\pm$1.04 mm. and the mean fibrin content is 0.3174 $\pm$0.0035 gram per 100 cc. of plasma. That an unmistakable relationship exists between the fibrin content and the sedimentation rate is shown by a correlation coefficient, derived from their data, of 0.459 $\pm$0.036. This serves to emphasize the fact that conclusions should be based upon mathematical constants, rather than upon general impressions.

DISCUSSION AND CONCLUSIONS

Table 2 summarizes the results found in this investigation.

Our results substantiate those of previous workers in respect to the significantly faster rate of sedimentation in women. This appears to hold for the adult state only, since such a sexual difference was not found in our previous work on girls and boys (1, 3). We plan to do further work to verify this point.

The number of red cells is significantly lower in women than in men. This is in accord with the results of previous investigators. In the present series there is unquestionably a relationship between the number of red cells and the sedimentation rate, since in both sexes a significant negative correlation was found. We have no explanation for the fact that the degree of correlation is lower in women than in the men.

The results found for the number of white cells, the percentage of neutrophiles, and the percentage of lymphocytes agree remarkably well in the two sexes. The rate of sedimentation is independent of these factors in the present series. Some physicians have expressed the opinion that the white count gives as much information regarding inflammatory conditions as the sedimentation test. We must conclude from this series that such an opinion is not yet substantiated. However, we shall test it further by investigations on febrile patients with higher leucocyte counts than the subjects in this series.

The relationship between the fibrin content and the rate of sedimentation is established by a significant positive correlation in both sexes.

Mathematical treatment of the present data permits us to conclude that as the red cells decrease in number, the sedimentation rate increases, and that as the fibrin content increases, the sedimentation rate increases. We

do not at present consider either factor causal. Much investigation of a physical-chemical nature must be done.

We desire to express our gratitude to Prof. J. Arthur Harris for his kindly advice and criticism in the preparation of this manuscript.

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THE RÔLE OF THE LIVER AND OTHER ABDOMINAL VISCERA IN THE DESTRUCTION OF EPINEPHRIN IN THE BODY

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It is a common observation that the action of epinephrin on blood pressure is of short duration even when given intravenously in large doses, the pressor effect being gone in about four minutes. Oliver and Schäfer (1894) noted this in their earliest experiments on the physiology of epinephrin. Although it can be easily demonstrated that the great bulk of the injected epinephrin has disappeared from the blood stream when the vasoconstriction has worn off, there is evidence that some of the drug persists in the blood stream when the usual circulatory phenomena accompanying its action are no longer present. Thus in the case of a rabbit whose superior cervical ganglion Joseph (1912) had excised approximately twenty-four hours previously, it was observed that the corresponding pupil attained its former size as late as forty-five minutes after the intravenous injection of 0.1 mgm. epinephrin for each kilogram of body weight. According to Jackson (1908), at the moment when the blood pressure has returned to normal it cannot be demonstrated by transfusion into another animal that the blood of the injected animal contains epinephrin. Trendelenburg (1910, 1916), using the method of perfusion at constant pressure through the hind limb of a frog, clearly showed that the disappearance of epinephrin from the blood stream paralleled the changes in the blood pressure as long as the injected doses of the drug were not massive enough to induce heart failure; under this condition the blood pressure could be normal when there was still a demonstrable quantity of epinephrin in the blood stream.

As regards the site of destruction of the epinephrin, Elliott (1905) stated there is general agreement that this is in the capillary bed everywhere in the animal body, with the exception of the lungs in which it has been impossible to demonstrate any loss of epinephrin on perfusion through the pulmonary circulation. Läwen (1904) found that after repeated transfusion of epinephrin through the hind limb of a frog a considerable amount disappeared. Carnot and Josserand (quoted by Trendelenburg, 1924) stated that the destruction of epinephrin was markedly accelerated by muscular exercise. Athanasiu and Langlois (1897) joined the portal vein and vena cava with a

Y-tube. They investigated the pressor action of epinephrin before and after excluding the liver from the circulation by clamping its vessels. In the former case the duration of the pressor effect was about two minutes, and in the latter about three minutes. Tatum (1912) incubated pieces of blood vessel with solutions of epinephrin, and found that these solutions lost markedly in potency as estimated by the frog perfusion method of Läwen; control solutions did not show such diminution.

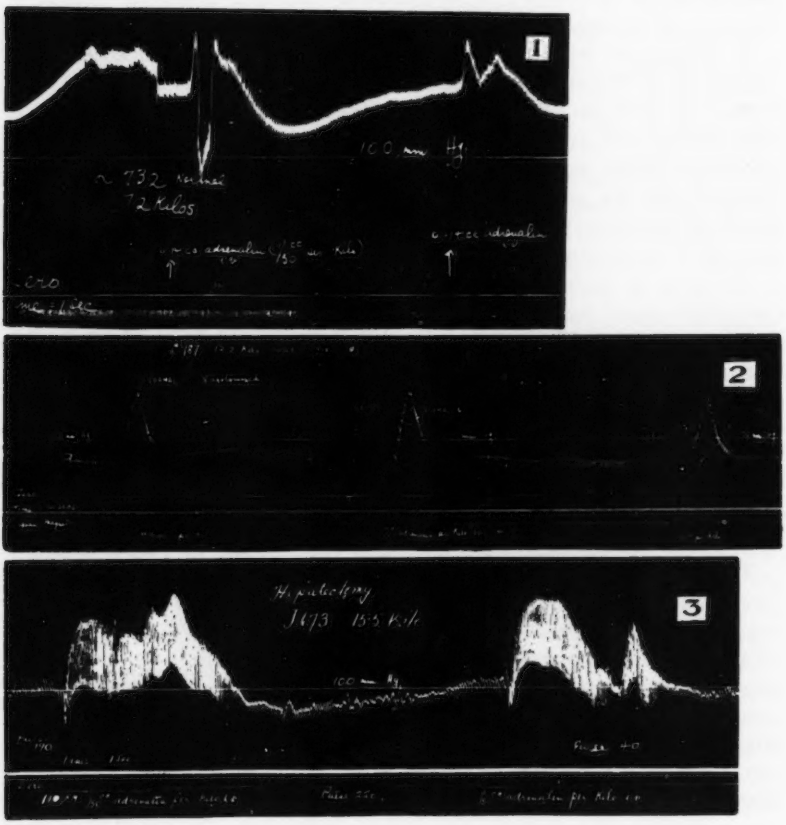
It is apparent from the foregoing data that the site of destruction of epinephrin in the animal body is universal, but what is not altogether clear is the proportionate rôle of the viscera, the muscles and the blood vessles in this process. Standard textbooks on pharmacology, for example that of Sollmann (1926), state that the liver is chiefly concerned in this process. The liverless dog as prepared in our laboratory is an ideal test object for deciding this point; we have, in addition, extended our observations to include the pressor effects of epinephrin in eviscerated preparations.

METHOD OF EXPERIMENT. Dogs were used and blood pressure estimations were made by introducing a cannula into the carotid artery under ether anesthesia. The results were almost always checked by at least one experiment performed under local anesthesia. When vagotomy was desired under local anesthesia the vagi were sectioned in the neck after the injection of a little procaine into them through a fine needle. Hepatectomy and total evisceration were performed by the usual technic. Fresh epinephrin (1:1000) was administered intravenously in doses of 0.02 cc. for each kilogram of body weight, the quantity being given in about 5 cc. of sodium chloride solution.

RESULTS OF EXPERIMENT. In confirmation of others we have found that the administration of the foregoing dose of epinephrin to a dog elicited a uniform pressor response with usually a characteristic notch in the curve (fig. 1). This pressor response was particularly uniform in the vagotomized animal in which, as is well known, the rise in blood pressure was much higher, although the duration of the pressor effect was not much greater. The more uniform response of the vagotomized animal to epinephrin is due to the elimination of the constantly occurring vagal inhibition accompanying the pressor effect of epinephrin. Occasionally, in the non-vagotomized animal the heart escapes from vagal inhibition so that the pressor effects are not as uniform as when the vagi are sectioned (fig. 2). The duration of the pressor effect in these preprations was about two minutes.

The administration of the same dose of epinephrin to a dehepatized dog is followed by a response which differs in two respects from the response of a normal dog. First, the action of epinephrin persists for about four minutes, and second, vagal inhibition is very marked, the pulse rate falling to about thirty-five a minute (fig. 3). It is difficult to determine whether

or not the blood pressure rises to a higher level in this preparation on account of the inertia of the mercury, since it may well be that with a slow pulse the systolic rise is much greater than it ought to be. For this reason the same dose of epinephrin was injected into a dehepatized dog in which the vagi were sectioned just before the blood pressure was recorded (fig. 4). It will



Figs. 1 to 3

be seen that the action of epinephrin not only lasts somewhat longer than in the normal animal (two and three-fourths minutes) but the pressor response is also definitely higher.

In view of the fact that certain workers have attempted to allocate the site of destruction of epinephrin to the gastro-intestinal tract we deemed it advisable to repeat this experiment on several eviscerated preparations

(fig. 5). Epinephrin has here a somewhat more marked and more lasting pressor effect, about five minutes.

COMMENT ON RESULTS. It is apparent from the foregoing observations that although the liver and intestines may play some part in the destruction of epinephrin, this part is no greater than can be accounted for by the removal of as much of the vascular tree as is contained in these viscera. At any rate, the site of destruction of epinephrin is preëminently neither in the liver nor in the intestines since the excision of these viscera does not

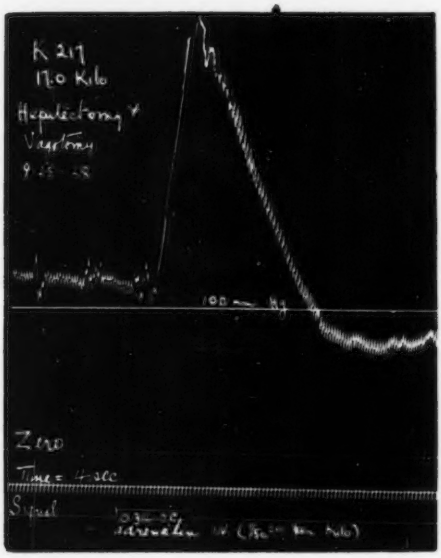
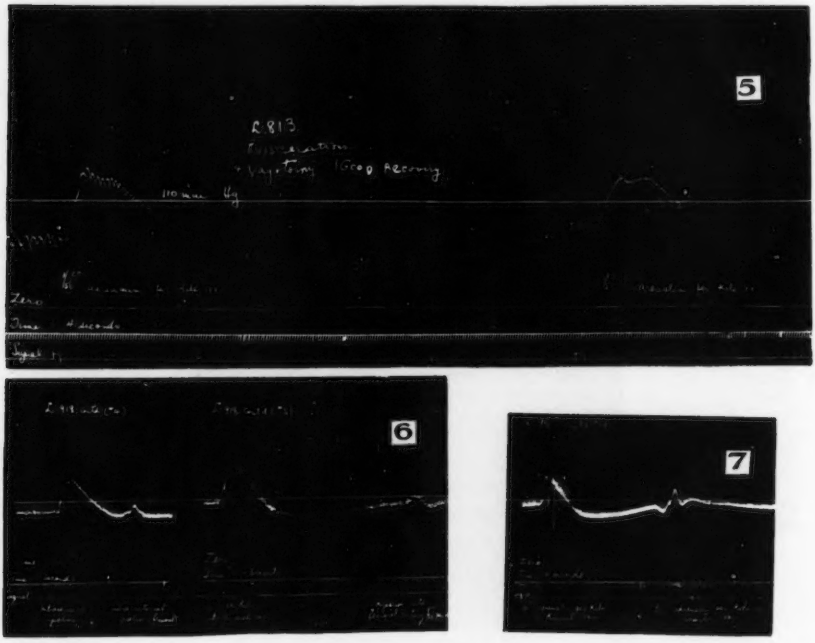


Fig. 4

strikingly prolong the pressor effect of the drug. This conclusion is a logical one. Since epinephrin acts on thoracic autonomic endings all over the body one would expect the most likely site for its destruction to be in these endings.

We have performed several experiments in which we compared the pressor effect of epinephrin injected into one of the mesenteric veins. The response was always much less than when the injection was into the jugular vein (confirming the work of Elliott, 1905). However, we believe that this experiment gives a false result, since the epinephrin reaches the general circulation in very much lower concentration. We mention this type of experiment only to condemn it. The injection of the standard

dose of epinephrin into the femoral artery similarly gives less response than when the injection is made into the femoral vein, but here again one may make the adverse criticism that the epinephrin is liberated into the general circulation at a very slow rate on account of the intense vasoconstriction which it induces (figs. 6 and 7).



Figs. 5 to 7

SUMMARY

A study was made of the pressor effects of epinephrin on normal, dehepatized and eviscerated preparations. Data are presented showing that in the absence of the abdominal viscera the organism still possesses such a marked capacity to destroy epinephrin as to make it unlikely that this destruction is a function of any particular viscus, but rather that it occurs all over the body. The conclusion is in harmony with the belief of Elliott that "adrenalin disappears in the tissues which it excites."

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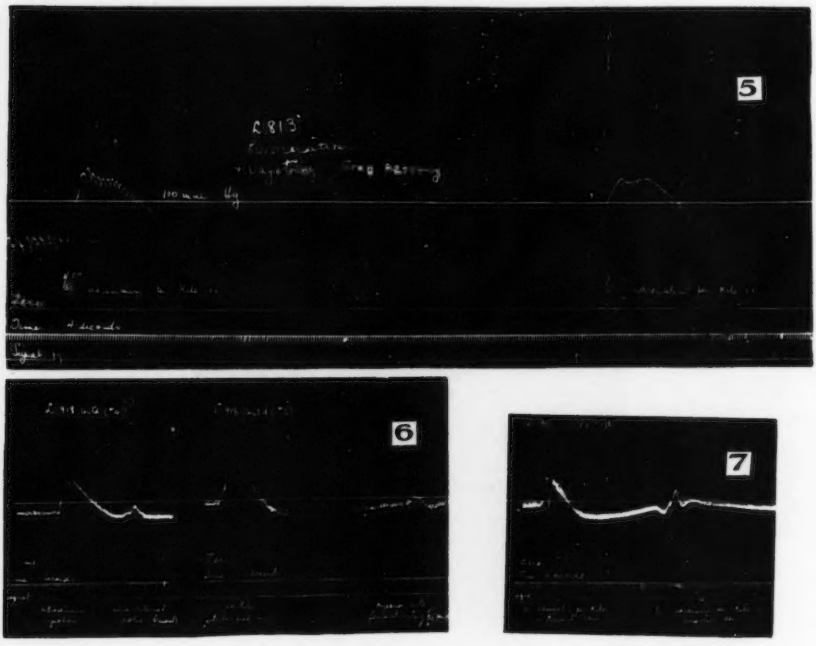

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A GRADIENT OF IRRITABILITY IN THE SMALL INTESTINE

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In 1913 Alvarez, while studying the absorption of gases in different parts of the small bowel, noted that the duodenum and jejunum were more irritable or more responsive to distention than the ileum. While attempting to measure this difference, he discovered the gradient in rate of rhythmic contraction (1), (2), and thereafter was too busy with other problems to return to the measurement of this first-noted gradient of irritability.

During the last year we have measured the threshold for faradic stimulation in four different parts of the small bowel of rabbits. The animals were anesthetized with urethane; the spinal cord distal to the fourth dorsal segment was pithed, and the abdomen was opened under warm saline solution.

One after another segments of bowel were gripped in the special electrode and clamp pictured in figure 1, and stimuli were thrown in. When the circular muscle was being used the electrode gripped a small area on the mesenteric border and directly opposite it there was fastened a tiny wire serrefine which was connected by a thread to a light lever writing on a drum. When the irritability of the longitudinal muscle was being studied the electrode was placed with its two wire points along the transverse axis of the gut. The faradic tetanizing current used was produced by a Porter inductorium connected to one dry cell.

We soon noted that when two electrodes were placed on the gut 5 cm. apart the irritability and amplitude of contraction at the caudad end of the loop was generally greater than that at the orad end. Experiments made in an effort to explain this phenomenon were not entirely satisfying because the results obtained were not always consistent, but the impression gained was that the irritation produced by the clamp at the caudad end of the loop lowered the irritability of the gut orad to it. In the experiments here reported two clamp-electrodes were placed 5 cm. apart, and the threshold was taken at the more sensitive end of the loop.

Figure 2, based on results obtained in twenty-five consecutive experiments, shows that in "normal" rabbits there is a remarkably constant gradient from duodenum to ileum. We put the word normal in quotation marks because so many of the animals available in the market are infested

with tapeworms and their cysts, coccidia, and cecal whipworms. The mean values for the four different regions are shown in table 1. The probable errors of the means are so small that, granting that the measure-

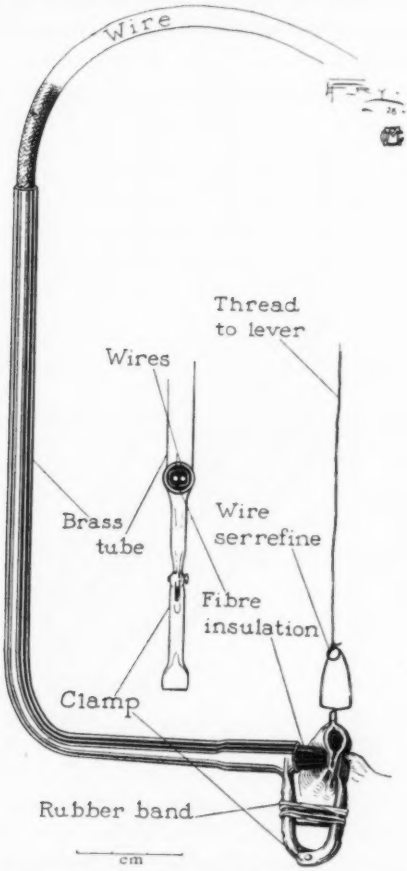

Fig. 1. Electrode and clamp. When in use it is clamped to the side of the tank of salt solution in which the animal lies.

ments are accurate, there can be no question about the presence of a gradient.

In order to see what effect if any the anesthetic had on the threshold, a few animals were prepared in the following way: Under procaine blocking, the cord was severed proximal to the fifth dorsal vertebra and the part

below this point was pithed. The abdomen was then opened and as one would expect with animals completely anesthetic distal to the upper part of the thorax, there never was any sign of distress and they lay quietly in the bath. The conclusion to be drawn from the figures obtained with these rabbits is that urethane had no effect on the irritability of the muscle (table 1).

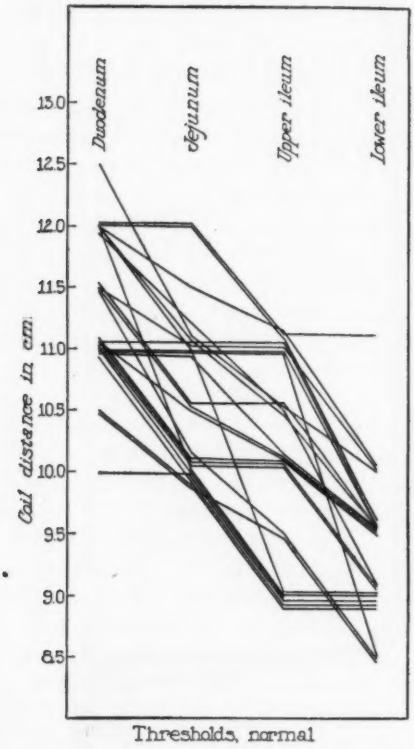

Fig. 2. Thresholds for faradic stimulation in four different parts of the small bowel of twenty-five normal rabbits. Nineteen were anesthetized with urethane and six by spinal transection.

A few experiments done with the longitudinal muscle indicated that its thresholds in the different parts of the gut are practically the same as those of the circular muscle.

So far we have done very few experiments with the galvanic current but in two animals we were able with it to demonstrate the gradient of irritability down the small bowel.

COMMENT. We believe it probable that the gradient in irritability that we have found is one of the important factors in determining the direction of normal peristalsis. In a paper soon to be published we will show that this gradient is reversed in animals in which injections of turpentine have produced necrotic areas about the ileocecal sphincter. So far as our data now go, it is reversed or flattened also in pregnant animals. When it is flattened peristaltic rushes are generally hard to start, spontaneous ones are absent or infrequent, and when they do appear they travel slowly and often come to a stop in the upper ileum. It must be admitted, however, that under the abnormal conditions of exposure in the tank of salt solution, the waves do sometimes rush on up to or beyond the irritated region in spite of the fact that the gradient of irritability is reversed. It is also hard to understand why with the gradient of irritability reversed and the gradient of latent period flattened, the gradient in rate of rhythmic contraction should generally remain normal. Apparently all the gradients are not, as Alvarez first suspected, equally dependent on or manifestations of some basic gradient of metabolism (3).

TABLE 1

Mean values of thresholds for faradic stimulation in different parts of the small intestine of rabbits

The figures represent coil-distance in centimeters

	ANIMALS	DUODENUM	JEJUNUM	UPPER ILEUM	LOWER ILEUM
Normal animals.....	25	Mean 11.4 ±0.1	10.7 ±0.1	10.2 ±0.1	9.5 ±0.1
		S.D.* 0.6	0.6	0.8	0.4
Urethane anesthesia...	19	Mean 11.4	10.7	10.2	9.4
Spinal transection.. ..	6	Mean 11.5	10.7	10.2	9.5

* S. D. represents *standard deviation*.

In all discussions of the relations between gradients and the direction of diastalsis (traveling waves) it must be remembered that although the flattening of one or more related gradients may make it easy for ripples or definite waves to run orad, it is still possible for food put into the stomach to travel caudad. Similarly ripples may travel north over the surface of a stream flowing south. Perhaps only with the marked reversal that comes with such conditions as hysteria, pregnancy, acute appendicitis or acute ileus do all the waves in the digestive tract for a time run orad.

SUMMARY

In the small bowel of the rabbit the irritability of the muscular coat is graded downward from duodenum to lower ileum. This gradient is prob-

ably of importance in directing normal diastalsis because when it is reversed by irritating the lower ileum, the rush waves are inhibited, slowed, brought to a stop, and sometimes even turned back.

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THE EXPERIMENTAL REVERSAL OF INTESTINAL GRADIENTS

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Several years ago Alvarez (1928) suggested that the diastaltic waves of the bowel probably follow gradients of rhythmicity, irritability, tone, latent period, muscular strength and metabolic rate. He suggested also that a reversal of these gradients might be effected by any lesion that would sufficiently raise the irritability and metabolic rate of the muscle at the lower end of the ileum. There was much clinical evidence at hand to show that peristalsis is reversed in the presence of such lesions but information was not available as to the condition of the gradients. Obviously the gradient theory would be much strengthened if, with the experimental reversal of one or more of the related gradients in the bowel, back-pressure or reverse peristalsis should be demonstrated.

In the course of our work during the last few years we have found two rabbits each with a small granuloma of parasitic origin in the wall of the ileum. In neither animal did this cause much mechanical obstruction but in one there was considerable interference with the progress of food residues, as shown by the fact that the bowel immediately above the tumor was contracted and empty, and the duodenum and upper jejunum were distended. When these animals were anesthetized with urethane, the spinal cord distal to the fourth dorsal segment destroyed, and the abdomen opened under a bath of warm physiologic salt solution, rush waves failed to appear spontaneously as they normally would do, and it was hard to start them in the usual way by injecting water into the esophagus.

The segment of bowel immediately above the lesion was then excised and placed in warm oxygenated Ringer's solution where it contracted rhythmically. In the animal that showed most obstruction the rate was from twenty to twenty-five a minute which is much faster than we have ever seen it in the ileum. There the rate is usually from nine to fifteen a minute. In this animal, then, the gradient of rhythmicity was decidedly reversed, but in the other one with a similar lesion it was normal. As will be seen later, the gradient in rhythmicity is probably not so good an index to the dynamic balance between the two ends of the gut as are some of the others such as the gradients of irritability and latent period.

[1] With the technical assistance in some of the experiments of Hugo Ascanio, Lucille Mahoney and Albon Overgard.

EXPERIMENTAL RESULTS. *The injection of irritants into the ileocecal region.* In a series of twenty-six experiments rabbits were anesthetized with ether or procaine, the abdomen was opened, and a few drops of turpentine or dilute phenol were injected into the sacculus rotundus, the mass of lymphoid tissue surrounding the ileocecal sphincter. Sometimes a little was injected also into a Peyer's patch. From one to five days later the animal was again opened under warm salt solution, and with the help of small wire clips which gripped the serous coat, several points along the small bowel were immobilized. More serrefines were attached opposite the others in such a way that, as loops of circular muscle contracted or relaxed, the motion was transmitted by threads to light levers writing on a drum. In this way records were obtained simultaneously from six or more regions, and the progress of peristaltic rushes was recorded.

The injection of the irritant material produced necrotic areas and sometimes small patches of peritonitis. The bowel for some distance above the injured region was almost always empty, and the duodenum and upper part of the jejunum were commonly distended with digestive residues. When the injury to the ileocecal region was sufficiently severe, the animals suffered with diarrhea and the colon and cecum were emptied.

Ordinarily when normal rabbits are opened and kept for several hours in the bath of salt solution, occasional peristaltic rushes are seen and many of them run the full length of the bowel, but in many of the animals with much irritation of the ileocecal region, rushes either failed to appear or else were infrequent. Only with difficulty could they be produced with the usual method of injecting water into the esophagus. These rushes often faded out after going a short distance or their advance was interfered with or blocked by ring-like contractions which appeared here and there.

Normally peristaltic rushes appear to "gather momentum" and travel faster in the ileum than in the duodenum. With a flattened gradient or one reversed at the caudad end one would expect the rushes to travel slowly especially in the lower part of the ileum, and actually, such slowing was often a striking feature in our records (table 1).

In a number of animals there were surprisingly few signs of back-pressure In some this was due to the fact that the lesion had not been made sufficiently irritating but in others the cause was not discernible. Perhaps in some the original gradient of forces down the gut was so steep or stable that it could not easily be reversed, and it may be that the possession of this type of gradient will account for the fact that there are men and women who in spite of the presence of such an irritating lesion as a ruptured appendix, can continue for several days to eat and digest with hardly a symptom of reverse peristalsis.

Occasionally, reverse waves ran orad a short distance from the irritable region. In one experiment the animal was pregnant; it aborted two days

after the injections were made into the ileum and just before it was opened in the tank. In this animal the back-pressure from the lower part of the bowel was so great that rushes could not be started, and at no time did the distended duodenum empty into the jejunum. Even the waves in the stomach faded out before they reached the pylorus, and some were reversed. The greatest amount of back-pressure was observed in animals opened from two to four days after the injection of the turpentine. After five days there were signs of a return to normal in the activities of the gut.

The local segmenting movements were sometimes active and sometimes quiet just as they are in "normal" animals.

TABLE 1

Percentage distributions showing rate of travel of peristaltic rushes in normal animals, animals with turpentine injected about the ileocecal sphincter, and animals with degenerative section of the vagi and later turpentine injected about the sphincter

CENTIMETER EACH SECOND	DUODENUM TO UPPER ILEUM			UPPER ILEUM TO TERMINAL ILEUM		
	Normal	Injured	Vagotomy and injury	Normal	Injured	Vagotomy and injury
1– 1.9		8	11		8	34
2– 2.9	10	8	23	3	11	32
3– 3.9	21	16	19	2	19	20
4– 4.9	24	12	16	12	11	6
5– 5.9	16	16	11	7	15	4
6– 6.9	11	8	11	10	13	4
7– 7.9	5	8	7	5	6	
8– 8.9	2	4		5	8	
9– 9.9		4		14	2	
10–10.9	3	4	4	5	2	
11–11.9	5	4		7	4	
12–12.9	2	4		3	2	
13–13.9	3			3		
14–14.9				9		
15–15.9				5		
16 or more		4		9		

For controls a few rabbits were etherized, the abdomen was opened and the intestines were handled. Two days later when these animals were opened again, the bowel behaved normally and many spontaneous rushes were seen.

WHAT BRINGS ABOUT THE BACK-PRESSURE? Having demonstrated that irritation of the lower part of the ileum will often produce back-pressure in the small bowel, with retardation or stoppage of peristaltic rushes, the next question was: What changes take place in the neuro-muscular mechanism of the gut to explain the phenomena observed?

Rate of rhythmic contraction. We were disappointed at so seldom being

able to demonstrate a reversal of the gradient of rate of rhythmic contraction. It was present in only two of the rabbits. In one, reversal was produced by a great slowing of the rate in the duodenum and in the other by a great acceleration in the rate of the lower part of the ileum. In the remainder of the experiments the rates were about normal.

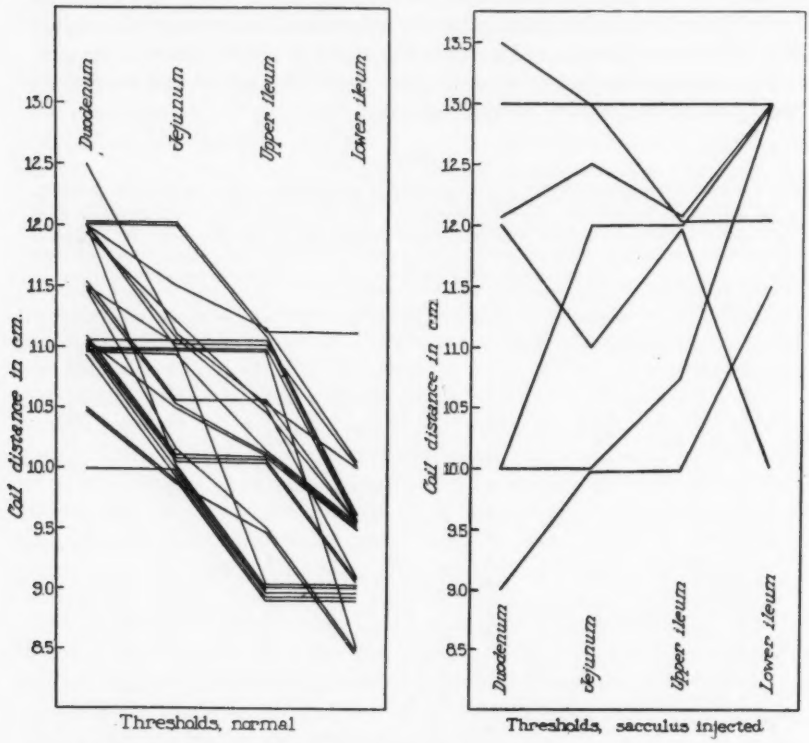

Fig. 1. Thresholds for faradic stimulation in four different parts of the small intestine of twenty-five normal animals and seven animals with turpentine injected about the ileocecal sphincter. Each line connects figures obtained with one animal.

From this and other studies it appears that the gradient of rate of rhythmic contraction is not so closely related to some basic gradient, perhaps of metabolism as Alvarez once thought. As we shall soon show, a better index to changes that have taken place in the muscular wall of the bowel will probably be found in the irritability of the muscle.

Thresholds for faradic stimulation. In this work we have used a specially devised electrode and clamp and have followed a technic described else-

where (1929a). The intact intestine was exposed in the tank of warm salt solution and stimulated with faradic tetanizing current supplied by a Porter inductorium energized by one dry cell. Figure 1 and table 2 show that as compared with the normal, there were decided differences in the irritability of the gut in the animals with chemical abscesses about the ileocecal sphincter. In most of them the whole bowel was unusually sensitive, but since the increase in irritability was more marked in the ileum than in the duodenum, and since in some animals the duodenum lost in irritability at the same time as the ileum gained, the usual gradient down the bowel was reversed. This reversal was the most striking and perhaps the most important observation made during the study. It alone might explain the tendency to reversal of peristalsis because it probably would be hard for waves starting in an insensitive region to travel toward a sensitive one. There were two animals with normal gradients of irritability, not shown in figure 1, but they were studied four

TABLE 2

Thresholds for faradic stimulation in four parts of the small intestine

The figures represent mean values in terms of distance in centimeters to the secondary coil of the inductorium.

TYPE OF ANIMAL	ANIMALS	DUODENUM	JEJUNUM	UPPER ILEUM	LOWER ILEUM
Normal	25	11.4	10.7	10.2	9.5
Sacculus injured	9	11.5	11.6	11.5	11.7
Vagi cut	11	11.6	11.1	10.9	10.2
Vagi cut and sacculus injured	2	12.0	12.5	12.5	12.5

and five days after the injection of the drug, when perhaps conditions in the ileum were somewhat restored to normal.

Latent period. Latent periods were measured with the technic to be described elsewhere (1929b). As will be seen from table 3, in twenty-five normal animals there was, with both faradic and galvanic stimulation, a definite gradation from short periods in the duodenum to longer ones in the ileum: a gradation which again would tend to keep peristaltic waves traveling from regions supplied with an active type of muscle to regions supplied with a more sluggish type.

In the animals with the injured sacculus faradic stimulation revealed a shortening of the latent period which was so much more marked in the ileum than in the duodenum that the gradient was flattened. For reasons that we do not understand these changes were not so marked with galvanic stimulation but it may be that more comparable results would have been obtained if further experiments had been done.

So far as latent periods go, then, the animals with the injured ileocecal

region still showed a slight gradient, with the duodenal muscle more efficient than the ileac, but this gradient was not so steep as in normal animals, and if we think of short latent periods as associated with higher irritability, we may say that the whole level of the values underlying the gradient was raised.

Conduction. When the bowel is stimulated at any point waves of contraction tend to run off in both directions, but the one traveling caudad,

TABLE 3

Mean latent periods in different parts of the small intestine expressed in decimal fractions of seconds

TYPE OF EXPERIMENT	TYPE OF ANIMAL	ANIMALS	OBSERVATIONS	DUODENUM	JEJUNUM	UPPER ILEUM	LOWER ILEUM	CONDITION OF GRADIENT	COMMENT
Intact bowel, faradic stimuli*	Normal	25	676	0.21	0.22	0.25	0.26		
	Sacculus injured	9	185	0.18	0.20	0.20	0.21	Fair	General increase in irritability
	Vagotomy	13	314	0.21	0.26	0.23	0.27	Up and down	Jejunum unusual
	Vagotomy, sacculus injured	3	53	0.29	0.23	0.20	0.21	Reversed	Ileum quick, duodenum slow
Excised segments, faradic stimuli	Normal	19	537	0.20	0.25	0.25	0.27	Good	Jejunum slow
	Sacculus injured	10	182	0.22	0.23	0.28	0.26	Good	
	Vagotomy	13	268	0.23	0.26	0.25	0.27	Fair	Duodenum slow
	Vagotomy, sacculus injured	1	20	0.20	0.20	0.26	0.20	Flat?	Only one animal
Intact bowel, galvanic stimuli	Normal	8	147	0.18	0.19	0.21	0.23	Good	
	Sacculus injured	2	41	0.18	0.16	0.17	0.23	Fair	
	Vagotomy	5	126	0.18	0.19	0.18	0.21	Flat	Ileum quick
	Vagotomy, sacculus injured	3	56	0.17	0.20	0.17	0.15	Reversed	Ileum remarkable
Excised segments, galvanic stimuli	Normal	6	151	0.18	0.19	0.22	0.25	Good	Duodenum unchanged
	Sacculus injured	3	60	0.16	0.20	0.23	0.24	Good	
	Vagotomy	5	112	0.18	0.19	0.20	0.21	Flat	Ileum quick
	Vagotomy, sacculus injured	1	23	0.20	0.18	0.20	0.20	Flat	Ileum quick, duodenum slow

* Secondary coil at 4 cm.

down the gradient, generally goes faster and farther than the one traveling orad against it. The rate of conduction is normally a little slower than that of the rushes, or from 1 to 5 cm. a second.

In some apparently normal animals it was hard to demonstrate the spread of conduction even 5 cm. away from a point stimulated, and a good idea of the irritability and conduction of a particular bowel could be obtained by keeping count of the number of measurable records obtained with each 100 stimuli. As will be seen in table 4 this number for the

intact duodenum was in normal rabbits 23.7, and in injured ones, 18.4. With the lower ileum, however, the corresponding figures were 21.3 and 38.2. Evidently, then, in the injured animals it was a little harder than normal to demonstrate conduction in the duodenum but considerably easier to demonstrate it in the lower part of the ileum.

In the animals with the injured sacculus the rates of conduction were found to be progressively slower from the duodenum to the terminal ileum (table 5). As one might expect with flattened gradients the rate orad tended to be faster than the rate caudad but on account of difficulties in making the measurements we cannot be sure about this point. The

TABLE 4

Measurable wavelets seen after 100 separate stimuli: an index to the ability of a particular bowel to conduct. Faradic stimuli used. Secondary coil at 4 cm.

TYPE OF ANIMAL	ANIMALS	OBSERVATIONS	DUODENUM	JEJUNUM	UPPER ILEUM	LOWER ILEUM	TOTAL	COMMENT
Normal, intact bowel	25	672	28.2	35.7	27.2	19.4	27.5	
Normal, excised bowel, 5 cm.*	19	520	51.2	52.0	37.7	46.0	46.8	
Normal, excised bowel, 10 cm			24.9	23.6	22.3	29.4	25.8	
Sacculus injured, intact bowel	9	240	18.4	38.6	35.0	38.2	32.9	Duodenum poor, ileum good
Sacculus injured, excised bowel, 5 cm	10	252	41.4	50.7	60.0	36.4	46.4	Unusual upper ileum
Sacculus injured, excised bowel, 10 cm			16.2	14.3	32.7	27.3	22.2	Conduction poor
Vagi cut, intact bowel	13	357	54.3	43.8	38.0	39.6	43.1	Much better than normal
Vagi cut, excised bowel, 5 cm	13	315	52.9	44.2	32.9	39.5	42.9	About normal
Vagi cut, excised bowel, 10 cm			25.8	42.9	32.9	23.7	31.1	Better than normal
Vagi cut, sacculus injured, intact bowel	3	53	0	40.0	12.5	29.4	20.7	Poor duodenum
Vagi cut, sacculus injured, excised bowel, 5 cm	1	20	33.3	0	0	40.0	20.0	One animal used
Vagi cut, sacculus injured, excised bowel, 10 cm			0	0	0	0	0	

* Two recorders were placed on each segment, one 5 cm. from the electrode and the other 10 cm. from it. A wavelet might reach the first recorder but not the second.

slowing in the rate of conduction in the lower part of the bowel can be shown by statistical methods to be almost certainly significant.

In thirty normal animals stimulation of the intact bowel 467 times produced 189 wavelets that traveled orad 5 cm. or more, and 278 that traveled the same distance caudad, which proves again what Alvarez and Starkweather (1919) showed years ago, that waves travel more easily caudad and go farther in that direction than they go orad (table 6). With a flattened gradient one would expect the waves to travel more easily orad than they do in normal animals but actually, in the intact bowel of the injured rabbits, there were twenty-six measurable waves traveling orad to sixty-one traveling caudad. The proportion was 1:2.3 as compared with the normal 1:1.5. So far as we can see now this finding does not fit with our theories in regard to gradients.

To sum up the observations on conduction: in the injured animals it was easier than normal to demonstrate conduction in the lower part of the ileum, but harder than normal to demonstrate it in the duodenum. The rate of conduction in injured animals was slower than normal. This slowing was most marked in waves traveling caudad. In the jejunum and upper ileum of the injured animals conduction orad was not easy to demonstrate.

The behavior of excised muscle. In order to differentiate, in the animals with the inflamed sacculus, the results of nervous inhibition from those of actual injury to muscle, segments from various parts of the bowel were excised and their behavior was studied in warm aërated Locke's solution. So far as rhythmic contraction went they behaved normally: the rates

TABLE 5

*Rate of conduction orad and caudad after stimulation with a faradic tetanizing current with the secondary coil at 4 cm.**

TYPE OF ANIMAL	ANIMALS	OBSERVATIONS	DUODENUM		JEJUNUM		UPPER ILEUM		LOWER ILEUM		TOTAL		COMMENT
			Orad	Caudad	Orad	Caudad	Orad	Caudad	Orad	Caudad	Orad	Caudad	
Normal, bowel intact	30	467	5.0	5.6	4.0	4.0	3.8	4.5	3.8	3.8	4.2	4.5	Rate faster caudad
Sacculus injured, bowel intact	9	87	5.1	4.0	2.7	2.3	2.7	3.0	1.8	1.3	3.1	2.3	Slow in ileum
Normal, excised segments	24	341	7.1	7.2	6.7	6.8	6.4	6.5	5.6	6.0	6.5	6.7	
Sacculus injured, excised segments	10	179	7.5	10.0	10.0	8.6	9.3	10.8	9.7	7.7	9.4	9.4	Faster than normal
Vagi cut, intact bowel	20	280	4.7	5.0	3.9	4.3	3.9	4.2	4.2	3.8	4.1	4.3	Normal
Vagi cut, excised segments	20	323	7.1	7.5	7.0	7.1	6.6	6.9	5.5	6.1	6.7	6.9	Normal
Vagi cut, sacculus injured, intact bowel	3	13	12.0		6.5	5.0		2.5	2.0	1.8	7.4	2.8	Slow ileum; slow conduction caudad
Vagi cut, sacculus injured, excised segments		4		4.5					5.5		5.5	4.5	

* The figures represent mean values in centimeters a second.

were normal and the amplitude good. The latent periods with either faradic or galvanic stimulation were not significantly different from those obtained with segments excised from normal animals (table 3).

Conduction, on the other hand, is normally much easier to demonstrate in excised segments than in the intact bowel, possibly because inhibition has been removed. This was true also in segments from the injured rabbits, except in the case of the lower part of the ileum, which, when excised, did not respond to stimulation with measurable wavelets any oftener than it did in the intact animals (table 4).

In segments of gut excised from normal animals wavelets could often be demonstrated running out 10 cm. or more from the point stimulated but in segments from injured animals this was seldom possible except in the case of the upper part of the ileum where conduction is normally very good.

Just as conduction is more easily demonstrated in excised segments of normal bowel, so also the rates of conduction are about one and one-half times those in the intact animal. The same was found true in segments removed from animals with the sacculus injured (table 5).

So far as we could see there was no marked difference between the behavior of excised segments from the animals with chemical irritation about the ileocecal sphincter and those from normal animals. The failure, therefore, of the gut to pass onward its contents could hardly have been due to injuries sustained by the muscle but was due more probably to

TABLE 6

The relative numbers of wavelets which traveled 5 cm. orad and caudad from point stimulated by a faradic tetanizing current, with the secondary coil at 4 cm.

	ANIMALS	OBSERVATIONS	DUODENUM		JEJUNUM		UPPER ILEUM		LOWER ILEUM		TOTAL	
			Orad	Caudad	Orad	Caudad	Orad	Caudad	Orad	Caudad	Orad	Caudad
Normal, intact bowel	30	467	43	68	48	75	47	66	51	69	189	278
Normal, excised segments	24	341	44	56	37	65	26	37	35	41	142	199
Ratio, orad to caudad, intact bowel			1 to 1.6		1 to 1.6		1 to 1.4		1 to 1.4		1 to 1.5	
Ratio, orad to caudad, excised segments			1 to 1.3		1 to 1.8		1 to 1.4		1 to 1.2		1 to 1.4	
Sacculus injured, intact bowel	9	87	8	2	3	17	5	21	10	21	26	61
Sacculus injured, excised segments	10	179	11	28	22	19	27	25	21	26	81	98
Ratio, orad to caudad, intact bowel			1 to 0.3		1 to 5.7		1 to 4.2		1 to 2.1		1 to 2.3	
Ratio, orad to caudad, excised segments			1 to 2.5		1 to 0.9		1 to 0.9		1 to 1.2		1 to 1.2	
Vagi cut, intact bowel	20	280	24	44	32	53	28	52	11	36	95	185
Vagi cut, excised segments	20	323	35	53	35	56	32	51	23	38	125	198
Ratio, orad to caudad, intact bowel			1 to 1.8		1 to 1.7		1 to 1.9		1 to 3.3		1 to 1.9	
Ratio, orad to caudad, excised segments			1 to 1.5		1 to 1.6		1 to 1.6		1 to 1.7		1 to 1.6	

nervous inhibition and to flattening or reversal of gradients of irritability and latent period.

Injury to the ileum after degenerative section of the vagi. The next problem was to determine if possible which is more important: nervous inhibition or upsets in gradients. It might well be that the back-pressure observed was due purely to inhibition produced at a distance by stimuli arising in the injured region. Since Johnson (1925) has shown that most of the myenteric plexus degenerates after section of the vagi, we decided to cut these nerves in a number of animals and, after waiting three weeks, to inject the irritant drug. If conduction along nerves is necessary for the production of stasis one would expect to see less back-pressure in animals that have lost most of these paths in mesentery and myenteric plexus.

TABLE 7

Experiments in which the vagi were cut and allowed to degenerate and then turpentine or phenol was injected around the ileocecal sphincter

NUMBER	TYPE OF VAGOTOMY	TIME ALLOWED FOR DEGENERATION	DRUG INJECTED	SUBSEQUENT INTERVAL	DIARRHEA	INJURY TO SACCULUS	APPEARANCE OF BOWEL	APPEARANCE OF STOMACH	RHYTHMIC ACTIVITY OF SMALL BOWEL	RUSHES	EXCISED SEGMENTS
		days		*days*							
1	Complete	30	Phenol	2	Severe	Slight	Ileum empty, duodenum and jejunum packed	Full	Quiet	Few and slow even in upper bowel	Contracted normally
2	Complete	21	Turpentine	2		Severe	Ileum empty	Small and flabby	Very active, rates normal	Very few and slow at first. Later many were induced	
3	Complete	30	Turpentine	2	Severe	Severe	Bowel empty, ileum a small hard cord	Small and flabby	Rates a little slow	Few, very slow	Contracted normally
4	Complete	31	Turpentine	3	Severe	Severe	Colon full of gas. Small bowel empty	Flabby	Active, rates a little slow	Few, very slow	
5	Possibly a few twigs uncut	21	Turpentine	2		Severe	Jejunum full. Some back-pressure		Rates a little slow	Easy to start but they travel slowly	Contracted well but very slowly
6	One vagus uncut	30	Phenol	2		Slight	Little back-pressure		Active, good gradient	Many, practically normal	Contracted well but duodenum and jejunum were very slow
7	Possibly some twigs left	30	Phenol	2	None	Severe	Some back-pressure	Flabby	Active, normal rates	Many with normal rate of travel	Contracted well but duodenum was very slow
8	Vagi and splanchnics cut	25	Turpentine	2	Severe	Severe	Bowel empty, very irritable. Cecum and colon active	Half empty, active. Ulcer near pylorus	Active, rates a little slow. Well graded	Many very slow; they fade out in the upper ileum	Small amplitude. Duodenum a little slow

We therefore studied four animals with complete vagotomy; one with vagotomy and splanchnicotomy; one with unilateral vagotomy; and two in which possibly a few twigs of one vagus were left (table 7).

Before proceeding to a discussion of the results obtained we must first note that in a preliminary study made to ascertain in rabbits the effects of vagotomy alone it was found that after this operation the bowel escaped from control and became very active. Rhythmic contractions took place incessantly, and rushes appeared after the slightest stimulus. These observations were made, of course, with the animal under urethane anesthesia and with the lower part of the cord destroyed so as to remove splanchnic inhibition.

Naturally with the brakes off and the bowel abnormally sensitive and active one would not expect stimuli from the ileum to inhibit or stop the rushes as well as they would in a quiet and more insensitive bowel, and this we believe explains our observation that after vagotomy, turpentine in the sacculus had less effect in preventing the appearance of rushes high up in the bowel. More of them started in the duodenum but more of them were slowed and stopped in the ileum (table 1). There was marked slowing of the rushes also in the one animal studied with a turpentine abscess made after degenerative section of the vagi and splanchnics. That this slowing was not due to the cutting of nerves alone was shown by preliminary studies.

We conclude, therefore, that something besides nervous inhibition holds back the progress of material down the bowel, and we suspect it is the reversal or flattening of gradients.

Gradients after vagotomy alone. Before the gradients could be studied in the animals with vagi cut and sacculus injured we had first to study the influence of vagotomy alone. Actually, as will be seen from the tables, the section and degeneration of these nerves had little effect. The gradient of irritability was a little flatter than normal and the figures suggest that the gradient of latent period was also changed. With faradic stimuli the jejunum appeared to be somewhat sluggish, but as this peculiarity of behavior could not be demonstrated with galvanic stimuli we are doubtful about it. The rates of rhythmic contraction in all segments tended to be slow but the gradation down the gut remained.

Gradients after vagotomy and injury to the sacculus. After degenerative section of the vagi, chemical irritation of the sacculus had no definite effect on the gradient of rhythmicity. The gradient of irritability was flattened by a marked lowering of the threshold (increase in coil distance) in the ileum, and for the same reason it was flattened also in the one animal with the vagus and the splanchnics cut.

In the three animals in which the latent period was studied the gradient (with both faradic and galvanic stimulation) was reversed on account of a

marked shortening of the interval in the lower part of the ileum. With faradic stimulation there was also a marked lengthening of the period in the duodenum.

In the excised bowel there was sometimes flattening of the gradient in rate of rhythmic contraction due to slowing of the rate in the duodenum. This was due probably to the vagotomy. The gradient of latent period also tended to be flattened on account of shortening of the interval in the ileum.

COMMENT. We know from much work on normal animals that the opening of the abdomen under salt solution and the destruction of the spinal cord distal to the fourth dorsal segment constitute such a strong stimulus to peristalsis that in a few hours the bowel will often empty itself. When, therefore, in the animals with the sacculus injured we did not see rushes during the first two or three hours of observation, we felt sure that in the intact animal there would have been even less chance of any transport of material from the stomach to the colon. Yet it must be remembered that the lumen of the bowel was unobstructed, the rhythmic movements were active, and the muscle was efficient.

It appears then that surgeons may have to find a new term for a type of ileus which is not due to mechanical obstruction or narrowing of the lumen of the bowel; it is not "paralytic" because the muscle is active and apparently in good working order, and it is not "dynamic" in the sense that there are none of those white ring-like contractions that sometimes obstruct the bowel in children. The explanation that we offer for it is that there has been a flattening or reversal of the normal gradient of forces and an excess of nervous inhibition. Perhaps until a better term is devised we may speak of a "flat gradient" ileus.

We believe that the gradient theory of peristalsis has been greatly strengthened by the experimental proof that irritation of the tissues at the lower end of the bowel can so raise the irritability of the ileac muscle and so shorten the latent period that the gradients of irritability and latent period are flattened or reversed.

The theory is still further strengthened by the fact that in animals with such flattened or reversed gradients, even after degenerative section of the extrinsic nerves of the small intestine, the rush waves are held back or slowed and the transportation of material through the bowel is impeded or stopped.

Unfortunately, so long as some nervous elements remain in bowel and mesentery there will be doubt as to the relative importance of reversed gradients and nervous inhibition in the production of stasis. That nervous inhibition still influences the bowel after degenerative section of the extrinsic nerves is indicated by the fact that when after vagotomy the gut was excised it appeared to escape from restraint much as it did after excision

from normal animals. As will be seen from tables 4 and 5 in segments excised after vagotomy there was no such increase in the number of measurable wavelets as is observed in normal animals because after the nerves were cut inhibition was removed and the increase took place in the intact bowel. Some restraint must still have remained, however, because after vagotomy the rate of conduction in the excised gut increased just as it did in segments from normal animals.

At first we were upset over the discovery that the various gradients are not always altered to the same degree: one may remain normal, another may be flattened and a third may be reversed, all in the same bowel. If they were all manifestations of some basic gradient of metabolism as Alvarez first suggested, we would expect them all to change together. Actually they do not do so and this observation, at first disturbing, now gives promise of being helpful because it may explain another puzzling fact which is that in man, reverse waves or ripples, producing symptoms of indigestion, appear to run orad from time to time over a stomach and bowel that can still transport material caudad.

SUMMARY

When considerable injury was produced in the tissues about the ileocecal sphincter of rabbits, they suffered from diarrhea and the colon was emptied. The ileum was emptied orad and food residues were held back in the duodenum. Peristaltic rushes were few; they were hard to start, and they were slowed and stopped in the lower part of the bowel.

After the injury the whole bowel was unusually sensitive to faradic stimuli, and in most of the experiments the normal gradient in irritability from duodenum to ileum was reversed. With the increased irritability of the bowel the latent periods were shortened, and the fact that this change was more marked in the lower part of the ileum than in the duodenum caused the normal gradient (of latent period) to be flattened.

Segments of gut excised from the injured animals and placed in warm aërated Locke's solution behaved normally, showing that the failure of the bowel to pass onward its contents was not due to injury to the muscle.

Chemical injury of the ileocecal region in animals with vagi and splanchnics cut and a large part of the conducting system in the bowel degenerated, still produced back-pressure in the small bowel and a marked slowing of rush waves. This suggests that the flattening of gradients had something to do with the failure of conduction.

Alvarez' law appears to be confirmed; namely, that irritation at any point in the bowel tends to slow the progress of material coming from the stomach toward it, and to hasten the progress of material moving caudad away from it.

The gradient theory of peristalsis appears to be strengthened.

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THE LATENT PERIOD OF INTESTINAL MUSCLE

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Thirteen years ago, after demonstrating graded differences in the latent period in muscle from different parts of the stomach, Alvarez (1916, 1917a, b) spent much time looking for similar differences in the behavior of the muscle from duodenum, jejunum, and ileum. Small strips were excised and suspended between tiny wire serrefines in a warm moist chamber; a tetanizing faradic current was led to the muscle by way of the serrefines, and records of the contractions were obtained with a light lever writing on a fast drum.

Unfortunately the results were not always clear-cut and therefore little was published about them. The persistence of rhythmic contractions made it difficult to secure a satisfactory base line, and often the first rise of the lever was so slow that it was impossible to say just when the contraction began. At other times the stimulus served only to modify the form or to increase the amplitude of a spontaneous contraction already present. Occasionally also the stimulus would produce a momentary depression followed by a rise. Repetition of the experiment and averaging of results did not always help because with currents strong enough to produce a quick contraction the muscle from dogs and cats often failed to relax completely and the latent period became progressively lengthened.

Attempts to get rid of rhythmic contractions by painting the muscle with drugs or by immersing it in poorly balanced salt solutions or isotonic solutions of non-electrolytes, such as glucose, were not successful because with the stoppage of rhythmic movements there went a decrease in irritability and in the amplitude of contraction. On account of these difficulties many of the measurements had to be discarded and a brief report was made only in regard to some of the more definite observations in dogs (Alvarez, 1922).

Results in dogs. The lines in figure 1 represent the gradation in latent period in muscle removed from several parts of the gut in normal and diseased dogs. Some of the abnormal dogs were killed on account of distemper and others died following some abdominal operation. The tetanizing current used was obtained from one dry cell connected to a Porter inductorium with the secondary coil at 4 cm. The measurements

were made on longitudinal muscle. In three animals the responses to stimulation of the circular muscle from the different regions of the gut were compared with those of the longitudinal and found to be a little slower.

In normal dogs the interval varied from about 0.15 second in the jejunum to about 0.30 second in the lower ileum. The duodenum was generally a little more sluggish than the jejunum, due perhaps to its greater sensitiveness to the trauma of excision and to the lack of oxygen. In sickly animals the gradient in latent period was flattened on account of a greater sluggishness of the muscle in the duodenum and a greater liveliness of that in the ileum.

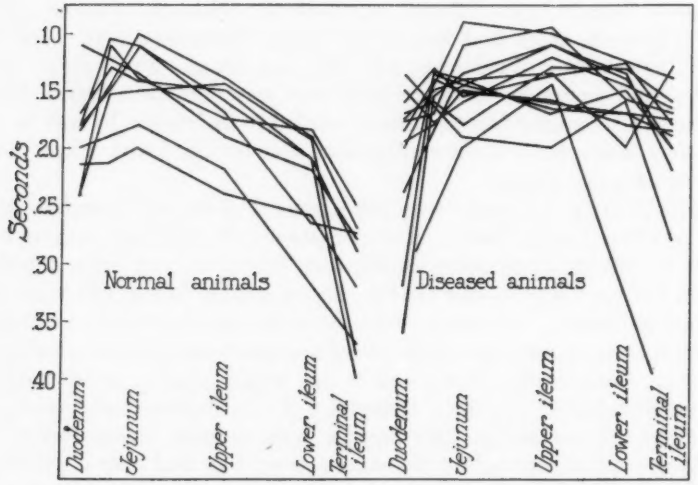

Fig. 1. Latent periods of muscle removed from different parts of the bowels of nine normal and fourteen diseased dogs. Lines connect data from one animal. The stimulus was a faradic tetanizing current.

Since it has been found that the shape of the contraction curve is related to other physiologic characteristics of a muscle, some studies were made on the duration of the rise and fall in the curves of contraction in different parts of the bowel of dogs, but constant differences were not found.

Results in cats. The latent period was studied in thirteen cats. One apparently normal animal showed a good gradient from 0.25 second in the duodenum to 0.32 second in the ileum, but two others that seemed healthy showed irregular, partly reversed gradients. One with diarrhea showed a steep gradient from 0.20 second in the jejunum to 0.48 second in the ileum. A pregnant animal showed a flattened gradient, 0.25 second in

the duodenum, and 0.29 second in the ileum. Four females which had aborted or littered three or four days previous to examination had markedly reversed gradients, about 0.27 second in the duodenum and about 0.18 second in the ileum.

It was interesting that the muscle from one cat which had aborted a few days previous to examination showed, immediately after death, latent periods of 0.36 second in the duodenum, 0.23 second in the jejunum, and 0.13 second in the ileum, and after twenty-four hours in the ice box, periods of 0.11 second in the jejunum and 0.22 second in the ileum. In the most healthy animals studied the duodenum was so much more sensitive to faradic shocks than the ileum that it responded well with the secondary coil at 8 cm. while the ileum responded only when it reached 4 cm.

It seems clear, then, that in cats just as in dogs, the gradient in latent period is flattened or reversed in sickly animals. Since all but two of the animals studied were infected with ascarides and tapeworms we cannot say what the gradient would have been in perfectly normal animals.

White rats. Studies made on nine white rats failed to show a constant gradation in either direction along the bowel. The length of the latent periods was about the same as in cats and dogs.

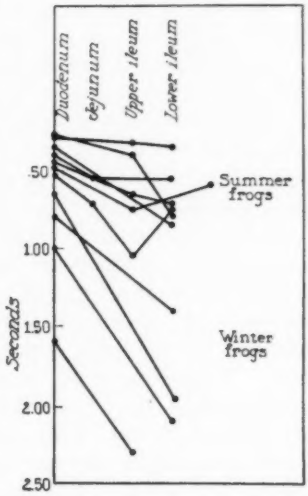

Fig. 2. Latent periods in different parts of the small bowel of eleven frogs.

Frogs. Figure 2 shows some fair and some good gradients in latent period obtained with the intestines of frogs. As was to be expected, the muscle was more sluggish in winter than in summer. This gradient was not apparent in some frogs studied by Gellhorn and Weidling (1926).

Rabbits. In excised segments from sixteen rabbits a constant gradation in latent period was not found. The periods were of about the same length as in dogs and cats. In one animal purged with cascara, there was a gradation from 0.13 second in the duodenum to 0.17 second in the jejunum, 0.19 second in the upper ileum, and 0.30 second in the lower ileum. As will be shown, a better technic for stimulating the bowel and for averaging the data obtained revealed the presence of a definite gradient in the small bowel of the rabbit.

Monkeys. Short strips of gastric and intestinal muscle were secured

from two macacus monkeys which died in the laboratory. In one the latent period was graded from cardia to pylorus in the stomach but was not graded in the bowel. The jejunum contracted after 0.60 second and the ileum after 0.50 second. In the other monkey the gradient of latent period in the stomach was reversed and that in the bowel was more as we thought it might be in a normal animal. The duodenum responded to stimuli with the coil at 6 cm. while the ileum responded only when it was moved in to 4 cm. The jejunum did not respond to any strength of current. The latent period in the duodenum was 0.32 second and in the ileum 0.64 second. These results, of course, were only suggestive.

Man. Bits of intestinal muscle were obtained twenty minutes after the execution by hanging, of a strong healthy man. As has been shown in more detail elsewhere, there was a suspicion of a gradient from short periods in the second portion of the duodenum to longer ones in the lower ileum. Muscle from the duodenal cap was very sluggish and that from the ileum next to the ileocolic sphincter was very lively.

More careful studies with the small intestine of the rabbit. Last year the measurement of intestinal latent periods was again attempted with the help of better methods and apparatus. Rabbits were anesthetized with urethane, the spinal cord distal to the fourth dorsal vertebra was pithed and the abdomen was opened under warm (38°C.) physiologic salt solution. Two points 5 cm. apart on the mesenteric border of the gut were each grasped by a specially designed clamp and electrode, described elsewhere (Alvarez, 1929); tiny serrefines were attached to the antimesenteric border opposite these clamps, and threads were run to light levers writing on a fast drum. In this way the latent period of the circular muscle was measured in duodenum, jejunum, upper ileum, and lower ileum. The stimulus was thrown in alternately at the upper and lower end of the loop so that one region could be recovering while the other was being tested.

First a series of records was made with a tetanizing faradic current as stimulus. The secondary coil was placed at 4 cm. because this appeared to give the best results with the least injury to the muscle. Then another set of records was made with a galvanic current as stimulus. The strength was usually about 5 milliamperes. Occasionally an attempt was made to reverse this sequence and to use the galvanic current first, but this did not work well. The passage of the galvanic current changed the tissue in some way so that it could not thereafter respond normally or at all to faradic stimuli.

Difficulties in measurement. The previously described difficulties in technic were again encountered, and oftentimes the rhythmic contractions were so persistent and troublesome that for a few minutes satisfactory work could not be done. Later, however, in all of the animals, after the

electric shocks had served somewhat to quiet the movements, reliable and easily measurable records were obtained.

When rhythmic contractions were present it was our custom always to throw in the stimulus as the lever reached the lowest point in its excursion. Although we saw little sign of rhythmic alternations in irritability or of a refractory period at any point in the cycle, it seemed wise to eliminate one possible variable in the conditions of the experiment.

Two components of the curves. Sudden alternations in the length of the latent period seemed often to be due to the fact that with faradic stimuli the curve of contraction was made up of two components. The appearance of the curves suggested the presence in the muscle of two types of fibers, one with a latent period between 0.1 and 0.3 second and the other with a period between 0.8 second and 2.0 seconds. Naturally when the first type of muscle failed to lift the lever away from the base line the latent period became that of the second type. We first thought that one muscle might be the longitudinal and the other the circular but a little study showed that this was not the case.

After doing considerable work on the problem it appears to us now that the best explanation for the first rise in the curves obtained with the faradic current is that it is due to direct stimulation of the muscle; the second sharp rise with the longer latent period is probably due to stimulation of the muscle by way of the enteric nervous system. In favor of this view is the fact that the second rise was never seen with galvanic stimuli, which have little if any effect on the nerves going to the bowel, and which apparently have no effect on the myenteric plexus because they do not give rise to traveling waves. The problem will be discussed in more detail in another paper.

Effect of one part of the gut on another a short distance away. As already mentioned, the measurements were made with two electrodes, one at each end of a loop of bowel 5 cm. long. First one end was stimulated and then the other. It was soon found that the caudad end of the loop was generally more sensitive; its threshold was lower, and the amplitude of contraction was greater than at the orad end. Occasionally, however, the reverse was true.

We spent some time trying to find an explanation for this, leaving one electrode stationary and moving the other toward and away from it on both the orad and caudad sides. Unfortunately the results obtained were not always clear-cut or consistent but the best ones indicated that when one electrode was moved caudad toward the other the contractions obtained by stimulating with the moving electrode diminished in amplitude and those obtained with the stationary one remained unchanged. Similarly when one electrode was moved orad, the amplitude of contraction at the orad, stationary electrode diminished. These results surprised us

because ordinarily conduction of stimuli along the gut is better caudad than orad. Under those circumstances we expected the trauma incident to the attachment of the orad clamp-electrode to affect the irritability of the bowel in the grip of the caudad one.

TABLE 1

*Distribution of latent periods obtained in several parts of the intact and excised bowels of rabbits**

TIME INTERVAL	DUODENUM				JEJUNUM				UPPER ILEUM				LOWER ILEUM			
	Intact		Excised		Intact		Excised		Intact		Excised		Intact		Excised	
	Records	Per cent	Records	Per cent	Records	Per cent	Records	Per cent	Records	Per cent	Records	Per cent	Records	Per cent	Records	Per cent
second																
0.1	31	18.7	33	24.1	18	11.1	9	6.3	8	4.4	5	3.9	5	3.1	2	1.6
0.2	97	58.5	71	51.8	99	60.7	79	54.9	109	60.3	77	59.3	83	50.0	61	48.4
0.3	33	19.9	30	21.9	35	21.5	43	29.8	39	21.5	30	23.1	54	32.5	41	32.6
0.4	5	3.1	3	2.2	11	6.8	9	6.3	24	13.3	14	10.8	23	13.9	19	15.1
0.5							2	1.4	1	0.6	3	2.3	1	0.6	3	2.4
0.6							2	1.4			1	0.8				
Total....	166		137		163		144		181		130		166		126	
Mean faradic...	0.21		0.20		0.22		0.25		0.25		0.25		0.26		0.27	
0.1	12	33.3	10	31.3	10	29.4	10	28.6	7	18.0	4	9.8	4	10.5	1	2.3
0.2	19	52.8	18	56.2	19	55.9	19	54.3	25	64.1	25	61.0	21	55.3	25	58.2
0.3	4	11.1	4	12.5	4	11.8	4	11.4	5	12.8	10	24.4	11	29.0	13	30.2
0.4	1	2.8			1	2.9	2	5.7	2	5.1	2	4.9	2	5.3	4	9.3
Total....	36		32		34		35		39		41		38		43	
Mean galvanic..	0.18		0.18		0.19		0.19		0.21		0.22		0.23		0.25	

* In the first group of data a faradic stimulus was used and in the second a galvanic stimulus was used. Contraction of the circular muscle was recorded. "Faradic intact" results are based on experiments with twenty-five animals; "faradic excised," nineteen; "galvanic intact," eight; and "galvanic excised," six. The percentages are shown graphically in figure 3.

Gradation in the latent period of circular muscle in the intact gut. The latent period of the rabbit's small bowel, in situ, with a faradic tetanizing current (with secondary coil at 4 cm.) varied from 0.1 to 0.6 second no matter what part of the bowel was used. Only when scores of measurements were gathered together into percentage distribution polygons and

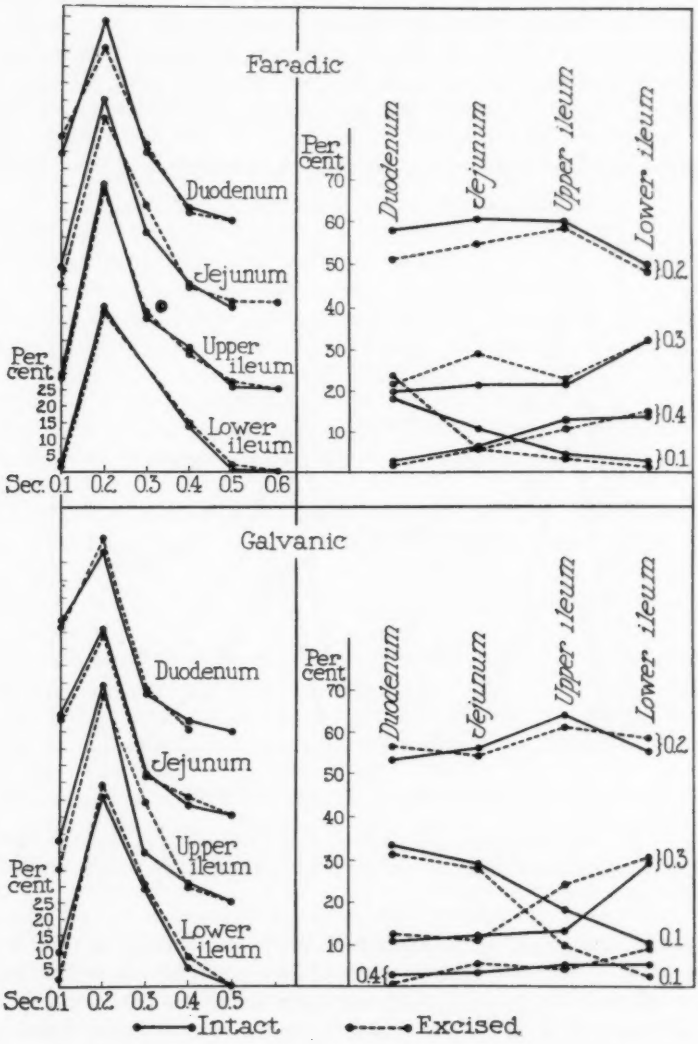

Fig. 3. The superimposed histograms on the left represent percentage distributions of the different short and long latent periods obtained in four parts of the bowel, intact and excised, with faradic and galvanic stimuli. The lines on the right show that as the percentage of short latent periods decreases from duodenum to lower ileum the percentage of long periods increases. The number of animals used and the actual figures are given in table 1.

their means calculated did the fact emerge that there is a gradation from the lively duodenum to the more sluggish ileum (table 1 and fig. 3).

In figure 3 it will be noted that with the faradic current the commonest measurement in all four regions of the bowel was 0.2 second, but that the number of 0.1 second periods was graded from the duodenum, in which they represented 18.7 per cent of the total, to the ileum where they represented only 3.1 per cent. The means in fractions of seconds were

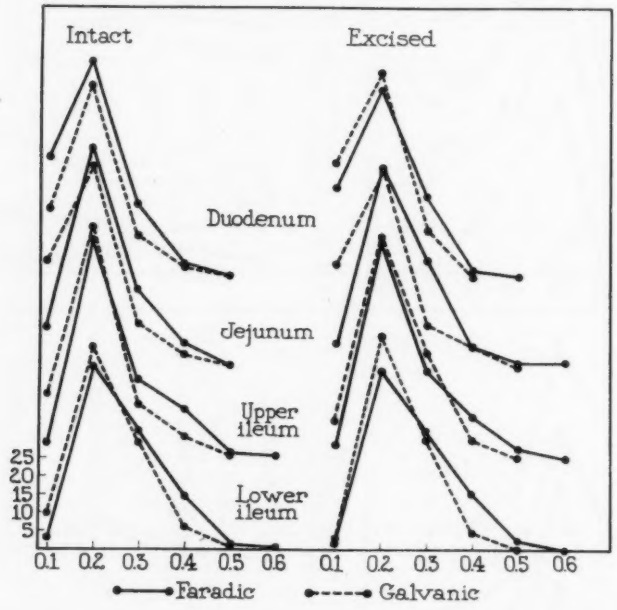

Fig. 4. The same histograms as in figure 3 but rearranged so as to show the differences in the distributions of the various short and long latent periods as obtained with faradic and galvanic stimuli.

as follows: duodenum, 0.21; jejunum, 0.22; upper ileum, 0.25; and lower ileum, 0.26. From the point of view of the statistician these figures are fairly trustworthy because they are each based on between 163 and 181 measurements and their probable errors vary between 0.004 and 0.005 second.

In computing these averages we first excluded those measurements which, on account of the presence of rhythmic contractions or on account of a slow rise in the first part of the curve, were indefinite. We excluded also all those in which the latent period appeared to be over 0.6 second

because it seemed obvious from the measurements made with clear-cut records that the true latent period was around 0.2 second, and nothing could be gained by including in the figures to be averaged those obtained at times when the muscle was fatigued or the strength of stimulus was weakened by some accidental leakage of current or by some increase in resistance.

We kept count of the number of the questionable records and were interested to find that in the intact bowel most of them were obtained with duodenum and lower ileum. Expressed as percentages of all the attempts at stimulation, the figures for duodenum, jejunum, upper ileum, and lower ileum were respectively 25.7, 7.0, 11.4, and 19.8.

Some of the difficulty in the duodenum may perhaps be ascribed to nervous inhibition, because with the excised segments the corresponding percentages of poor records were 10.5, 17.5, 17.7, and 18.2. We are left, however, with no explanation for the increase in the number of poor records with excised jejunum and upper ileum. The percentage of poor records in all regions of the intact bowel was 16.3 and in all excised segments, 19.1.

Latent periods with galvanic stimulation in the intact gut. As will be seen from table 1 and figure 4, the results obtained with the galvanic current were similar to those obtained with the faradic. The most marked difference was in the number of 0.1 second reactions in duodenum and jejunum.

The means, in fractions of seconds, were as follows: duodenum, 0.18; jejunum, 0.19; upper ileum, 0.21, and lower ileum, 0.23. These figures are all shorter than those obtained with the faradic current. The percentage of 0.1 second latent periods was graded from thirty-three in the duodenum to eleven in the lower ileum.

There was less doubt about these figures than there was about those obtained with faradic stimuli because there were so few long or questionable reactions. With the galvanic stimulus the bowel either contracted promptly or not at all.

Latent periods in the intact gut of animals anesthetized by spinal transection. In order to see what effect if any the anesthetic had on the latent period, a few animals were prepared in the following way. Under procaine blocking the spinal cord was severed distal to the fourth dorsal vertebra and the part below this point was pithed. The abdomen was then opened and as one would expect with animals completely anesthetic distal to the fourth dorsal segment, they lay quietly in the bath without any sign of discomfort.

Table 2 shows that while urethane (in doses of 2 gm. for each kilogram of body weight) had no effect on the latent period of the lower end of the ileum, it greatly lengthened that of the duodenum, and it slightly length-

TABLE 2

*Distributions of latent periods obtained in several parts of the bowel with and without urethane anesthesia**

LENGTH OF LATENT PERIOD	DUODENUM				JEJUNUM				UPPER ILEUM				LOWER ILEUM				TOTAL	
	With urethane		Without urethane		With urethane		Without urethane		With urethane		Without urethane		With urethane		Without urethane			
	Records	Per cent	Records	Per cent	Records	Per cent	Records	Per cent	Records	Per cent	Records	Per cent	Records	Per cent	Records	Per cent	With urethane	Without urethane
second																		
0.1	13	10.7	17	43.6	8	6.4	7	22.6	5	3.3	2	9.1	1	0.8	3	7.7	27	29
0.2	76	62.3	17	43.6	83	66.4	16	51.7	93	61.2	13	59.1	65	52.9	16	41.0	317	62
0.3	29	23.8	4	10.3	26	20.8	5	16.1	30	19.7	6	27.3	40	32.5	14	35.9	125	29
0.4	4	3.3	1	2.6	8	6.4	3	9.7	23	15.1	1	4.5	16	13.0	6	15.4	51	11
0.5									1	0.7			1	0.8			2	
Total.....	122		39		125		31		152		22		123		39		522	131
Mean.....	0.22		0.17		0.23		0.21		0.25		0.23		0.26		0.26		0.24	0.22

* A faradic current was used as stimulus. The secondary coil was at 4 cm. This study is based on measurements made on eighteen urethanized and six non-urethanized rabbits.

ened that of the jejunum and the upper part of the ileum. This gradation in the depressant effect of urethane on the muscle of the bowel seems to fit perfectly with the observations of Child on cyanides. It fits particularly well with the observations of Alvarez and Starkweather who placed excised segments of duodenum, jejunum, and ileum in dilute solutions of potassium cyanide, which interferes with oxidation, and found that the duodenum with its fast metabolic rate was paralyzed, the jejunum with its intermediate rate was somewhat inhibited, and the ileum with its slow rate was unaffected.

We observed some difference in the latent periods of the muscle in segments *excised* from urethanized and non-urethanized animals but will attempt no explanation of them because the experiments on non-urethanized animals were few and the probable errors of the means correspondingly large. For the same reason we will not attempt to discuss the results of galvanic stimulation in these animals.

Latent periods of muscle in excised segments of gut. In most of the experiments as soon as the necessary records had been secured with the intact bowel the animal was killed (by puncturing the diaphragm) and segments were removed from duodenum, jejunum, upper ileum, and lower ileum. They were placed one by one in a glass baking dish containing aërated Gasser's (1926) solution at 38°C. Each loop of bowel was gripped at its two ends as before with the same clamping electrodes, and the same faradic and galvanic currents were used.

We were surprised to find that in spite of the less favorable conditions outside the body, the latent periods were practically the same as they were in the intact bowel, and the gradation was the same (fig. 3).

SUMMARY

A gradation in latent period of muscular contraction with short intervals in the duodenum and longer ones in the ileum has been demonstrated in the small intestine of several types of laboratory animals. There was little if any change in the latent period when the bowel was excised and placed in warm aërated balanced saline solution.

In the small bowel of the rabbit faradic tetanic stimulation, after an interval of approximately 0.2 second, usually produced a contraction in which the lever rose slowly for 1 or 2 seconds and then rose rapidly. With galvanic stimuli the lever rose rapidly from the start and the resultant curve was different from that obtained with faradic stimuli. The latent period was a little shorter than that observed with the faradic current.

The presence of two components in the curve of contraction obtained with faradic stimuli but not with galvanic seems best explained by the

assumption that the first rise is due to a direct effect on the muscle and the second to an effect transmitted by the nervous system.

The presence of a clamp pinching the wall of the bowel influenced the irritability of the muscle 5 cm. orad to it.

Urethane anesthesia had no effect on the latent period of the muscle in the lower part of the ileum but it lengthened that in duodenum and jejunum.

The gradient of latent period may perhaps help to determine the direction of normal diastalsis; it is suggestive that it was found flattened or reversed in several distempered dogs and a few pregnant cats.

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CHANGES IN THE SERUM CALCIUM OF CATS DURING FASTING

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In a previous investigation it has been shown by one of us (1) that the serum calcium level of fasting dogs is maintained practically constant until an advanced stage of inanition has been reached. At that time the calcium concentration diminishes. Meglitzky (2) reports that in fasting cats the serum calcium likewise tends to fall below the initial level in the late phase of fasting (unfortunately, Meglitzky does not record the actual loss in weight of his animals) but during the earlier stages he observed

TABLE 1

CAT NUMBER	INITIAL WEIGHT	LOSS IN WEIGHT		DAYS OF FASTING	Ca CEREBRO-SPINAL FLUID	CALCIUM IN SERUM		
		Kilograms	Per cent			Normal	Loss in body weight	
							Below 20 per cent	Above 20 per cent
	kgm.				*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	2.70	0.22	8.2	14	5.6	9.60	*10.60*	
2	3.10	0.45	14.5	14	5.2	11.40	11.40	
3	2.20				5.3	10.70		
4	2.94					11.00		
5	2.80	0.70	25.0	11		10.60		10.40
6	4.40	0.85	19.3	11		10.70	*11.20*	
		1.39	31.6	18				11.40
7	2.47	0.38	15.4	9		11.80	11.50	
		0.62	25.1	16				10.80
8	2.27	0.40	17.6	9		11.30	11.50	
		0.63	23.4	16				11.50
9	3.25	0.67	20.0	9		10.60	10.60	
		1.02	31.4	16				9.90

most extraordinary increases in the calcium, ranging from 32 to 99 per cent of the initial value in the first 14 to 16 days of starvation. We wish to place on record our own results obtained in experiments with fasting cats because they are so contrary to Meglitzky's findings. From our data, presented in the table 1, it can be seen that such a change in the

serum calcium concentration of fasting cats as is described by Meglitzky is practically non-existent. In one cat (no. 6) the serum calcium has risen about 5 per cent before the loss in weight reached 20 per cent, and about 6.5 per cent in the more advanced stage, and in another cat (no. 1) the serum calcium rose 10 per cent during an early phase of the fast. In this cat, no. 1, however, the initial serum calcium concentration was very low, namely, 9.6 mgm. per cent. In the matter of the serum calcium changes, therefore, our findings on the cat are not different from those previously reported for fasting dogs. In all the other cats we studied the serum calcium remained unchanged during the early fasting periods, and diminished only in the more advanced stages.

Another serious discrepancy is manifest in our results and those of Meglitzky pertaining to the initial serum calcium concentration in the cat. We find that in the normal cat the serum calcium is on the average 10.9 mgm. per cent. Baumann and Kurland (3) give as the average of their analyses 10.2 mgm. per cent, while Meglitzky reports normal serum calcium values ranging from 12.3 to 16.2 mgm. per cent, or an average of 13.4 mgm. per cent.

In both investigations the cats received water during the whole time of the fasting experiment. The analytical procedure was likewise the same; namely, Clark's method.

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STUDIES ON SUPRARENAL INSUFFICIENCY

IV. The Blood Sugar in Suprarenalectomized Rats

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It is well known that in cases of Addison's disease and in suprarenalectomized animals the blood sugar may fall to a low level. In nearly half of the cases of adrenal failure studied in the clinics of the Evans Memorial, the blood sugar level was found to be below the lower normal limit (Rowe, 1928). Other recent workers who have observed low blood sugar levels in Addison's disease are Myers (1920) and Marañón, Carrasco and Soler (1925).

Banting and Gairns (1926) noted a terminal fall in blood sugar in suprarenalectomized dogs, but they concluded that the suprarenal cortex does not play a direct part in carbohydrate metabolism. Rogoff and Stewart (1926) also observed in dogs an inconstant, moderate, terminal fall in blood sugar, which they regarded merely as concomitant with a moribund condition.

Swingle (1927) found that in suprarenalectomized cats a drop in blood sugar begins about twenty hours after recovery from the anesthetic and continues slowly until death. He concluded that the muscular asthenia and progressive fall in body temperature which are typical symptoms of suprarenal insufficiency in cats may be attributed in part at least to the decrease in blood sugar, and that hypoglycemia may be one cause of death; not the sole cause, however, since the animals die eventually in spite of glucose injections. Yonkman (1928) reported similar findings in dogs and drew similar conclusions regarding the early symptoms. Hartman, MacArthur, Gunn, Hartman and MacDonald (1927) found a lowering of the blood sugar in suprarenalectomized cats similar to that reported by Swingle.

The observations cited above were made on species which die of acute suprarenal insufficiency within a comparatively short time after operation. The albino rat, however, may live for a comparatively long time after double suprarenalectomy, exhibiting meanwhile a chronic type of insufficiency. This extended survival is usually attributed to the presence in the rat of accessory cortical material which eventually may become

exhausted. Since a study of suprarenal insufficiency in the rat is in progress in this laboratory it seemed pertinent to investigate the blood sugar in a series of rats exhibiting various degrees of insufficiency.

Kuriyama (1918), Catan, Houssay and Mazzocco (1920) and Artundo (1927) found that the blood sugar content of suprarenalectomized rats was on the average slightly lower than that of their controls. The blood sugar did not fall below the normal range and tended to return to the normal average value within a period of a few weeks after operation. Cori and Cori (1927) found the blood sugar values for single determinations on six fasting suprarenalectomized rats to be from 53 to 79 mgm. per 100 cc. They gave 99 mgm. as the average value for fasting normal rats. None of these authors, in dealing with rats, give detailed information concerning the degree of suprarenal insufficiency in their experimental animals. The conclusions of some of these investigators may also be open to criticism, in that they were based on determinations made during the first week after operation.

METHODS. A mixed stock of colored varieties of the albino rat was used. The selection of animals, care of the animal room, diet, post-operative care, and the technique of suprarenalectomy have been described in previous papers from this laboratory (Belding and Wyman, 1926; Wyman, 1928a). Approximately equal numbers of each sex were used in each experiment. No sex differences with respect to blood sugar were noted. Rats from three to six months old were selected for suprarenalectomy. Autoplastic cortical transplants were made by the technique of Jaffe and Plavska (1926) in young rats from one and a half to four months old. A description of the technique, of the results of the operation, and of the microscopic appearance of the transplants has been given in a previous paper (Wyman, 1928b). One point of technique seems worthy of note. In a series of 39 successful transplants made for this and for other studies the capsule was carefully stripped from one suprarenal gland which was transplanted in two pieces on the left side of the abdomen. The gland which was transplanted on the right side was cut into two pieces without removing the capsule. This was done with the idea that removal of the capsule might favor the growth of the transplant. It was found at autopsy that in 38 cases one or two large masses of cortical tissue had regenerated from the gland with the undisturbed capsule, while with the exception of five cases the gland without the capsule had completely disappeared. Evidently removal of the capsule is unfavorable to successful transplantation of the suprarenal.

Blood sugar was determined by the ferricyanide micro-method of Folin (1928) using 0.05 cc. capillary pipettes of the type described by Folin for collection of blood samples. In a preliminary experimental series frequent excessively high blood sugar values were observed, for which no adequate explanation was at hand. By preparing the dilute tungstic acid and the

dilute sugar standard fresh each day, these aberrant observations were eliminated from the later experiments upon which the present paper is based. The source of this error has been found by Folin (1929) to be the deterioration of the tungstic acid solution. It should be noted in comparing our figures with those of other investigators, that the Folin micromethod gives blood sugar values from 5 per cent to 15 per cent lower than the Folin-Wu method.

Blood for sugar determinations was taken from 24 hour fasting rats by neatly clipping off the tip of the tail with a sharp knife and immediately receiving the drop of blood in the 0.05 cc. capillary pipette. After taking the blood the tip of the tail was dipped into a solution of adrenalin or Liquor Ferri Subsulphatis U. S. P. IX to lessen bleeding, and then the cut tip was sealed with collodion. The rats were not anesthetized and were confined only by holding them in the hands. They had been gentled before beginning the series of determinations and did not show fear or other emotion when they were picked up in preparation for clipping the tail.

In most cases blood was taken for sugar determinations once a week throughout the experiment, in a few cases at shorter intervals. Suprarenalectomized rats were allowed to rest undisturbed for about two weeks after operation before the first post-operative blood sample was taken.

Suprarenalectomy. Blood sugar determinations were made before and after double suprarenalectomy in a series of nineteen rats. Before operation the blood sugar values varied from 61 to 110 mgm. per 100 cc. whole blood. The average figure is 82 mgm. (51 determinations). Of these values 37 (72.6 per cent) lie between 70 and 100 mgm., which may be considered as the usual range of blood sugar values. Of the remaining fourteen cases, seven are between 60 and 70 mgm., and seven are between 100 and 110 mgm. (see table 1).

Following operation, five rats died of subacute suprarenal insufficiency within a period of 14 to 29 days.[1] In addition to these five, two more died with symptoms of insufficiency, 43 and 44 days after operation. This group of seven will be considered as a unit, demonstrating terminal stages of suprarenal insufficiency. In one case no blood samples were obtained before death. In five cases, coincident with the appearance of definite symptoms of insufficiency (bloody exudate around eyes and nose, low temperature, asthenia) from two to five days before death (for a period of 15 days in one case) the blood sugar was between 50 and 60 mgm. In two cases the blood sugar determinations were made during the extreme terminal stages of suprarenal insufficiency. In addition to the above symptoms these animals had diarrhea and frequent slight convulsions,

[1] The terminology indicating the degree of insufficiency as shown by the time of survival after operation is that of Jaffe (1926).

TABLE 1

RAT NUMBER	PRE-OPERATIVE BLOOD SUGAR				POST-OPERATIVE BLOOD SUGAR										REMARKS
4	90	63	77	DS	53										Died 16 NA
17	94	101		DS	83	36	(terminal stages)								Died 20 NA
20	77	105	83	DS	62	61									Died 25 NA
1	87	70	84	DS	64	83	59								Died 29 NA
10	91	78	74	DS	78	83	55	51	69	63					Died 43 NA
2		70	65	DS			65	118	63	30	(terminal stages)				Died 44 NA
15	99	105		DS	78	93	91	80	75	72	73	89	80	80	K 97 NA
3	73	80	63	DS	54	67	73		69	63	63	62	57	64	K 99 NA
5	110	87	75	DS	59	76	69	81	73	77	64	77	65	70	K 104 NA
6	88	89	95	DS	63	92	70	75	65	72	67	79	72	80	K 104 NA
7	74	73	61	DS	48	83	68	81		63	65		64	84	K 104 NA
13	77	102	74	DS	88	91	90	73	70	67	62	73	63	68	K 97 NA
14			61	DS	74	78	63	64	58	65	55	63	50	68	K 97 NA
8	87	81	71	DS	72	92	70	77	86	70	69	87	70	80	K 104 CA
11	75	78	89	DS	85	88	81	91	73	70			73	84	K 140 CA
12	72	83	80	DS	68	76	72	88	78	63	73				K 140 CA
16	85	103	91	DS	70	94	93	76			78	90	88	95	K 140 CA
19	87	109		DS	78	102	91	110	92	78	88	79	88	82	K 140 CA
40	85	85	81	CO	83		88	97	84	115	76	75	74	83	
					69	72	82	76	73	104					K 98
50	105	77	87	CO	82	115	85	93	90	108	92	81	80	96	
					85	83	84	72	87	86	106				K 112

Blood sugar values are in milligrams per 100 cc. whole blood. With the exception of rats 40 and 50, the determinations are approximately weekly and the first post-operative determination was made about two weeks after operation. In rats 40 and 50 the determinations were made at intervals of from 2 to 5 days, and the first post-operative determinations were made 4 (no. 40) and 5 (no. 50) days after operation. The letters have the following meanings: DS, double suprarenalectomy; CO, control blank operation; K, killed for autopsy, with number of days after operation; NA, no accessory suprarenal tissue found at autopsy; CA, gross accessory cortical tissue found at autopsy. The first six rats in the table (nos. 4 to 2 incl.) died of suprarenal insufficiency. In rats 10 and 2 the last determination was made one day after the preceding determination. The next seven rats (nos. 15 to 14 incl.) had no accessory tissue and may be considered chronically insufficient. The next five rats (nos. 8 to 19 incl.) had gross accessory cortical tissue and were biologically normal with respect to cortical function.

increasing as time of death approached. Blood sugar was 30 and 36 mgm. respectively, about 12 hours before death. The autopsy findings in all cases were those characteristic of death from acute or subacute suprarenal insufficiency (see Wyman, 1928c).

A second group of this series (7 rats) were killed at 97 to 104 days after operation, and no gross accessory cortical tissue was found at autopsy. These rats had shown no marked symptoms of insufficiency. The changes in weight since operation had varied from a loss of 8 grams to a gain of 75. At autopsy, with the exception of one case, these rats were found to have little or no abdominal fat. They may be regarded as having chronic suprarenal insufficiency. One rat of this group did not deviate from the normal range of blood sugar values. In the other six rats a fall in blood sugar level was observed, 14 to 34 days after operation. From this time on the blood sugar values remained at the low normal level (60 to 70 mgm.) or below (down to 48 mgm.).[2]

A third group (5 rats) were killed at 104 or 140 days after operation, and were found to possess gross accessory cortical tissue (verified by microscopic examination of sections). The accessory masses were from 4 to 6 mm. in diameter. These rats had gained from 43 to 104 grams since operation. At autopsy all were found to have abundant abdominal fat. These rats showed no deviation from the normal range in blood sugar values. In the total number (43) of determinations on this group only three values below 70 mgm. were found (69, 68, 63).

Autoplastic cortical transplantation. Blood sugar determinations were made on nine rats from 49 to 79 days after autoplastic transplantation of both suprarenal glands. Three determinations were made on each rat, at intervals of one week. Immediately after the last determination the rats were killed for autopsy and in all cases one or two well vascularized masses of cortical tissue were found at the sites of transplantation. Individual masses were from 0.5 to 5 mm. in diameter. The results of microscopic examination of these masses were the same as reported in a previous communication (Wyman, 1928b). In no case was there any evidence of the presence of chromaffin tissue. In one case a gross accessory mass of cortical tissue (3 mm. diameter) was found in the left adrenal site. In all cases the rats appeared to be healthy, had gained considerable weight since operation (98 to 210 grams), and at autopsy were found to have abundant abdominal fat. The blood sugar values were all well within the normal range, varying from 71 to 112 mgm., with a mean value of 88.

Control operation. In a group of five rats a series of blood sugar determinations was made before and after a control operation consisting of

[2] In the preliminary experimental series mentioned under *methods* the blood sugar in one case fell to the lower normal range 35 days after operation and remained there until death from chronic insufficiency occurred at 74 days after operation.

exposure and handling of both suprarenal glands and the removal of a piece of fat from near each suprarenal site (see table 1). Before operation 4 to 7 determinations were made on each rat, and after operation 15 or 17 determinations were made during a period of from 56 to 68 days. Later these rats were killed for autopsy. Since operation they had been in good condition and had gained weight (52 to 140 grams). At autopsy abundant abdominal fat was found and the adrenal glands appeared normal. In each case the blood sugar values exhibited a normal variation throughout the experiment. No abnormally low values were observed, the lowest being 69 mgm.

In two other rats blood sugar determinations were made before and for 20 days after a control operation. During this period the blood sugar varied normally. At the end of the 20 day period double suprarenalectomy was performed on each. Following the latter operation, in one case the blood sugar showed a normal variation during a period of 28 days. The rat was in good condition and had gained 26 grams since the second operation. At autopsy abundant abdominal fat was found, together with a gross accessory cortical mass, 3 mm. in diameter, in the left suprarenal site. In the other case, 14 days after suprarenalectomy the blood sugar level fell to the lower part of the normal range and the rat began to show symptoms of insufficiency (low temperature, diarrhea, sluggishness, ruffled hair). Seven days later more severe symptoms appeared (bloody exudate from eyes, asthenia, loss of appetite) and the blood sugar value fell below 60 mgm. (59, 51). Two days after the last determination the rat died, and the findings typical of subacute suprarenal insufficiency were seen at autopsy.

Discussion. Blood sugar determinations on normal unoperated rats indicate that in the rat a blood sugar value below 60 mgm. per 100 cc. (by the Folin micro-method) may be considered abnormal. Following double suprarenalectomy values below this level are frequently observed. The decrease in blood sugar level parallels the severity of the symptoms of suprarenal insufficiency. In rats with chronic insufficiency and showing no marked symptoms the blood sugar is maintained at a fairly constant level, but this level is in the lowest portion of the range of normal values and occasionally falls below the normal low limit. It is worthy of note that marked hypoglycemia does not occur until the terminal stages appear. The normal blood sugar found in rats possessing either gross accessory cortical tissue or successful cortical transplants (in the absence of demonstrable chromaffin tissue) indicates that the low blood sugar observed after double suprarenalectomy is associated with cortical insufficiency. That the results observed after suprarenalectomy are not produced by operative trauma is demonstrated by the maintained normal blood sugar level following control blank operation. This is especially well emphasized in

the case described above, in which a control operation was followed later by double suprarenalectomy, with subsequent death of the animal from subacute insufficiency.

Swingle (1927) suggested that the suprarenal cortex supplies some hormone which probably acts as a check upon the secretory activity of the islets of Langerhans.

There is a great mass of literature dealing with the relation of the suprarenal medulla and its secretion, adrenalin, to carbohydrate metabolism. An extended criticism of the earlier work may be found in the review by Stewart (1924). Recently, Britton, Geiling and Calvery (1928) demonstrated that after medulliadrenal inactivation cats suffer a severe and persistent lowered ability to counteract the effects of minute amounts of insulin. Whatever the mechanism involved, it appears that under emergency conditions the suprarenal medulla is an important factor in maintaining the blood sugar level. The results reported in this paper indicate that under ordinary conditions the medulla is not essential for maintaining a normal blood sugar level. Both parts of the suprarenal gland, however, have a possible relation to carbohydrate regulation. The part played by the cortex, be it direct or indirect, appears to be concerned in the steady maintenance of a normal blood sugar level under ordinary conditions, possibly in the manner suggested by Swingle. The part played by the medulla is an important adjunct for rapid adjustment under emergency conditions, as shown by Cannon and his colleagues.

SUMMARY

1. The normal blood sugar range in the rat, by the Folin micro-method, is found to be from 61 to 110 mgm. per 100 cc. whole blood, with an average value of 82 mgm.

2. Following double suprarenalectomy, coincident with the appearance of marked symptoms of subacute insufficiency, the blood sugar values fall to between 50 and 60 mgm. In the terminal convulsive stages they may be as low as 30 mgm.

3. In rats with chronic suprarenal insufficiency, but showing no marked symptoms, the blood sugar may fall to the lowest portion of the range of normal values and be maintained at that level, occasionally dropping below the normal low limit.

4. In rats possessing either gross accessory cortical tissue or successful cortical transplants, in the absence of demonstrable chromaffin tissue, the blood sugar remains within the normal range.

5. The blood sugar is normal following control blank operation, showing that the results observed after suprarenalectomy are not produced by operative trauma.

6. The results indicate that the low blood sugar observed after supra-

renalectomy is associated with cortical insufficiency. It is suggested that the cortex is concerned in the steady maintenance of a normal blood sugar level, while the medulla is an important adjunct for rapid adjustment under emergency conditions.

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IV. CONTRIBUTIONS TO THE PHYSIOLOGY OF THE PANCREAS

The Elimination of Dyes in the External Secretion of the Pancreas

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Oldberg and Ivy (1927) tested the excretory ability of the pancreas toward 32 dyes, and made a preliminary report. Subsequently Crandall (1928) reported briefly upon 62 dyes, some of which were duplications of the work of Oldberg and Ivy. More recently we have extended this series in an attempt to analyze the action of the pancreas as a dye excreting mechanism and to obtain a dye which might serve as a function test for the pancreas. In all, 142 tests have been made of 90 different dyes; 10 of these dyes have been found to be eliminated by the pancreas.

Method. Dogs were used exclusively. Using aseptic technique, the main pancreatic duct was cannulated and a Rehfuss tube attached to the cannula; this tube was coiled in the abdomen and the free end brought out through a stab wound. A rubber toy balloon was fastened to the free end and the balloon closed with a rubber stopper; the capacity of the balloon was 40 cc., which was usually sufficient to take care of the over-night secretion. Animals prepared in this way will secrete copiously for several weeks; in most cases, the period of usefulness is in the neighborhood of two weeks.

All of the dyes were administered intravenously. From 30 to 200 mgm. of dye were injected in from 10 to 30 cc. of water or physiologic salt solution. In some instances, dilute NaOH was used as the solvent, since some dyes are insoluble in water or saline. Prior to the administration of the dyes, pancreatic secretion was stimulated by giving food or water. The juice was collected at hourly or half-hourly intervals for 2 hours and then at irregular intervals for 12 hours. Color always appeared in the pancreatic secretion within an hour after the injection, if the pancreas was able to eliminate the dye. Therefore, if no dye appeared within two hours following an injection, a second dye was administered.

When the dye used was one which gives a more intense color in an acid medium than in an alkaline, the juice was acidified. Tests for leuco bases were not made except in a few instances. The dyes used are listed alpha-

betically in table 1, together with their color index numbers, toxicity, and inhibitory effect on pancreatic secretion. Schultz numbers are not used for the reason that the color index list of dyes is more complete than that of Schultz. Most of the dyes are commonly referred to by many synonyms; an effort was made in listing the dyes to choose the most common name, in any case the color index number will leave no doubt as to what dye was used. If the dyes are not listed in the color index, we use the chemical

TABLE 1

Dyes which were not excreted by the pancreas

Numbers refer to Color Index; toxicity and inhibition of secretion noted where present

Acid Blue 707; Acid Brown 239, death in ½ hour, complete inhibition of secretion; Acid Green G 666; Acid Violet 698; Alizarin 1027; Alizarin Blue S 1067; Alizarin Green G 917; Alizarin Red 1034; Alizarin Yellow R 40, slight inhibition of secretion; Alkali Blue 703; Amaranth 184; Anilin Blue 689; Anilin Yellow 137; Benzopurpurin 4B 448; Biebrich Scarlet 280; Bismark Brown Y 331; Bordeau Red 88; Brilliant Cresyl Blue 877; Brilliant Green 662, slight toxicity; Bromsulphalein; Carminate, Sodium 1239; Chrysoidin R 21; Chrysoidin Y 20; Congo Red 370; Cresyl Violet; Eosin, Bluish 771; Eosin Y 768; Erythrosin R 772; Ethyl Orange; Fuchsin Red 667; Gentian Violet 680; Hoffman Violet 679; Indigo Carmine 1180; Indocyanin B; Iodine Green 686, death in two minutes; Isorosindulin I; Isorosindulin II; Janus Green B 133, slight toxicity; Janus Red 266, slight toxicity, slight inhibition of secretion; Lacmoid 908; Light Green SF 670; Magdala Red 857; Malachite Green 657; Martius Yellow 9; Meldola's Blue 909; Meta-cresol-sulphonephthalein; Methyl Blue 706, death in five minutes; Methylene Green 924; Methylene Violet (Bernthsen); Napthol Green B 5; Napthol Yellow 10; Neutral Blue 832, marked toxicity, slight inhibition of secretion; Neutral Red 825; Neutral Violet 826; New Methylene Blue N 927, moderate toxicity; Orange G 27; Orange I 150; Orange II 151; Orange IV 143; Orcein 1242, slight toxicity; Ortho-cresol-sulphonphthalein; Patént Blue 712; Phenol-sulphonephthalein; Phloxine 774; Ponceau 3R 80; Pyrol Blue; Pyronin B 741; Rose Bengal 777; Rosindulin HCl 829, slight inhibition of secretion; Rosolic Acid 724, slight toxicity; Safranin Extra Bluish 842, slight toxicity; Thionin 920; Thymol-sulphonephthalein 50; Toluidine Blue O 925; Tropaeolin O 148; Trypan Blue 477; Trypan Red 438; Vital Red HR 456; Wool Fast Blue 833.

Note: Methylene blue was injected 7 times; 3 of these injections were followed by excretion of the dye in the pancreatic juice while in 4 cases there was no excretion.

terminology if known, otherwise, the most common name of the dye is employed.

Dosage of dyes must necessarily have only a limited significance, unless given in terms of pure dye substance. As received from the manufacturer, dyes commonly contain from 10 to 60 per cent of inert material, usually sodium chloride or dextrin. Since we are interested at present only in the permeability of the pancreas and not in quantitative measurements, we are presenting our dosages in terms of crude dye, in the form in which it is

ordinarily sold. Where possible, dyes which have been approved by the Commission on Standardization of Biological Stains were procured; in these cases the manufacturer lists the total dye content on the label.

Results. It will be noted that of the 90 dyes used, 10 appear in the pancreatic secretion. The amount of dye injected does not seem to be a major factor in determining amount of excretion, within the limits of dosage used by us. The injection of 100 mgm. of dye ordinarily does not result in a pancreatic secretion twice as deeply stained as that which follows the injection of 50 mgm.

Approximations of the concentration of dye in the pancreatic secretion were made, using three of the more readily excreted dyes, namely, rhodamin B, safranin extra bluish, and parafuchsin. Rhodamin B was injected intravenously to the amount of 100 mgm., and the concentration of dye in the pancreatic secretion estimated by comparing samples of the secretion with known concentrations of dye in pancreatic juice. The dog weighed 16.3 kilos, and assuming the blood volume to be 5.5 per cent of the body weight, it may be calculated that immediately after injection the blood contained 1 mgm. of dye per 9 cc. If we estimate the total body fluids to be 85 per cent of the body weight, then if the dye diffused equally throughout all the body fluids, its concentration would still be far higher than that in the pancreatic secretion, namely, 1 mgm. per 140 cc. of body fluids.

Safranin extra bluish, also known as methylene violet RRA, was used in the same manner, 75 mgm. being injected. The maximum concentration appearing in the pancreatic secretion was 1 mgm. per 1000 cc. It was calculated that immediately after injection the concentration of the dye in the blood was 1 mgm. per 12 cc., if it diffused equally throughout the body fluids, the concentration of the dye would be approximately 1 mgm. per 185 cc. of fluid. After an injection of 50 mgm. of parafuchsin the corresponding figures were found to be 1 mgm. per 1600 cc. for pancreatic secretion (actual measurement), 1 mgm. per 18 cc. of blood (calculated), and 1 mgm. per 280 cc. of body fluids (calculated).

Sodium thiocyanate is apparently more readily excreted by the pancreas. An injection of 100 mgm. of thiocyanate was made subcutaneously, and the amount present in the pancreatic juice was estimated by comparing the color developed in the juice after acidifying, centrifuging, and adding 2 drops of 10 per cent ferric chloride, with the color of similarly treated pancreatic secretion to which sodium thiocyanate had been added in known amounts. Concentrations of thiocyanate below 1 mgm. per 400 cc. could not be detected. The highest concentration reached after the subcutaneous injection of 100 mgm. of thiocyanate was 1 mgm. per 100 cc. pancreatic juice. The calculated concentration of thiocyanate in body fluids if uniformly distributed would be 1 mgm. per 128 cc.

Iodine was not found in the pancreatic juice after the intravenous injection of 250 mgm. of sodium iodide. Pyridium (phenylazo-alpha-alpha-diamino-pyridine hydrochloride) was injected in two animals using a dose of 100 mgm.; in neither case did color appear in the pancreatic secretion.

An interesting observation incidental to the main purpose of our work was the excretion of safranin O and phloxine from the mucous membrane of the colon. Tests were made because the foregoing dyes colored the stool in animals to which they were administered. These two dyes were administered to colon pouch dogs and found to be present in washings from the colon.

It should be noted that certain dyes may be detected in the pancreatic juice only after acidification, since their color is weak or absent in an alkaline medium. These are acid fuchsin, fast yellow, methyl orange and tropaeolin O.

Discussion. We find no common property in the dyes excreted by the pancreas. Matsuo (1928) reports that of the 72 dyes used in his laboratory the three (uranin, eosin AG and eosin W yellow) which were excreted by the pancreas all belonged to the xanthene group and all were of acid reaction. We also find uranin to be excreted. The color index lists no dyes under the names eosin AG and eosin W yellow. We have injected eosin Y (C.I. 768) on three different occasions in different animals but can find no trace of this pigment in the pancreatic juice. Of the ten dyes in the xanthene group which we used, only uranin (fluorescein) and rhodamin B are excreted; rhodamin B is a basic dye. Ziegler and Mendel (1927) state that the three dyes excreted by the pancreas in their experiments (amaranth, Martius yellow, and methyl violet) are of unlike chemical structure, but note that all three are water soluble.

Table 2 lists the excreted dyes according to chemical structure, following the classification of the color index which is similar to that of Schultz. It will be noted that the ten dyes which appear in the pancreatic secretion belong to five distinct chemical groups. It may possibly be of significance that both safranin O and safranin extra bluish are excreted, as well as rhodamin B which is closely related to the safranins. In this connection it is interesting to recall that the azines (Marston, 1923) have the property of precipitating trypsin. Of further interest is the observation that, in general, the excreted dyes are of rather simple chemical structure.

Table 3 lists the ten dyes which we found to be excreted by the pancreas, according to their solubilities in water, alcohol, ether, and olive oil; table 4 classifies them as to acid and basic properties, and table 5 according to rate of dialysis through a collodion membrane. Since the same dye may vary in properties when obtained from different sources, solubilities and rate of dialysis were determined by us, in each instance, on the same lot

from which material was taken for injection. For determination of rate of dialysis 5 cc. of a 0.15 per cent solution of dye in distilled water was dialyzed in a collodion bag against 50 cc. of distilled water. Congo red, which according to Freundlich (1922) is a colloidal dye, was included in this series as a control. Fluorescein when used as a saturated solution (less than 0.15 per cent) in distilled water showed no dialysis in 6 hours,

TABLE 2

Excreted dyes classified according to the color index

(Color index number given first, common name second)

Mon-azo dyes
- No. 16, Fast Yellow
- No. 142, Methyl Orange

Triphenylmethane and diphenylnaphthylmethane dyes
- No. 676, Para-Fuchsin
- No. 678, Basic Fuchsin
- No. 692, Acid Fuschin (slightly toxic)

Xanthene dyes
- No. 749, Rhodamin B
- No. 766, Fluorescein

Azine dyes
- No. 841, Safranin O (slightly toxic)
- No. 842, Safranin Extra Bluish (slightly toxic)

Thiazine dyes
- No. 922, Methylene Blue

TABLE 3

Acid or basic properties of excreted dyes

ACID DYES	BASIC DYES
Acid Fuchsin	Basic Fuchsin
Fast Yellow	Methylene Blue
Fluorescein	Para-Fuchsin
Methyl Orange	Rhodamin B
	Safranin O
	Safranin Extra Bluish

but when the solution was made slightly alkaline with NaOH this substance dialyzed as readily as any of the other dyes.

For a time we felt that molecular size might be a factor in determining which substances would be excreted by the pancreas, but in view of the fact that sodium iodide (or the iodide ion) did not appear in the pancreatic secretion although it passes readily into most of the body fluids, this factor does not seem to be of much importance. Some of the excreted dyes are acid and some are basic; they vary in solubility although all are water solu-

ble; without exception all of them dialyze readily. However, many crystalloidal, water soluble dyes of low molecular weight were not eliminated by the pancreas. We find no physical or chemical properties on the basis of which one can predict which dyes will be excreted by the pancreas.

No dye appears in the pancreatic juice in any considerable concentration. Safranin O, safranin extra bluish, and parafuchsin stain the juice most deeply. The maximum concentration in which these dyes were found in the pancreatic secretion was from $\frac{1}{5}$ to $\frac{1}{10}$ of that which they were calculated to attain in the body, on the assumption that they diffused equally throughout all the body fluids. This assumption is probably unjustified, which would make the disparity between the concentration of dye reaching the pancreas and that excreted in the pancreatic juice still greater. We are forced to the conclusion that the pancreas is very impermeable to

TABLE 4

Solubilities of excreted dyes

DYE	WATER	95 PER CENT ALCOHOL	ETHER	OLIVE OIL
Acid Fuchsin	Sol.	Sol.	Sl. Sol.	Insol.
Basic Fuchsin	Sol.	Sol.	Insol.	Insol.
Fast Yellow	Sol.	Sl. Sol.	Sl. Sol.	Sl. Sol.
Fluorescein	Sl. Sol.	Sol.	Sl. Sol.	V. Sl. Sol.
Methyl Orange	Sl. Sol.	Sl. Sol.	V. Sl. Sol.	Sl. Sol.
Methylene Blue	Sol.	Sol.	Insol.	V. Sl. Sol.
Para-Fuchsin	Sol.	Sol.	Sl. Sol.	Insol.
Rhodamin B	Sol.	V. Sl. Sol.	Sl. Sol.	Sl. Sol.
Safranin O	Sol.	Sol.	Sl. Sol.	Sl. Sol.
Safranin Extra Bluish	Sol.	Sol.	Sl. Sol.	V. Sl. Sol.

TABLE 5

Per cent of dye found in dialyzate when 5 cc. of 0.15 per cent solution in distilled water were dialyzed through collodion against 50 cc. distilled water for 2 hours

Acid Fuchsin	44 per cent
Basic Fuchsin	24 per cent
Fast Yellow	46 per cent
Fluorescein	0.0 in 6 hours
Fluorescein (in dilute NaOH)	48 per cent
Methyl Orange	51 per cent
Methylene Blue	28 per cent
Para-Fuchsin	48 per cent
Rhodamin B	16 per cent
Safranin O	44 per cent
Safranin Extra Bluish	38 per cent
Congo Red (Colloidal control)	0.0 in 6 hours

organic dyes, not eliminating the greater number and permitting the passage of only a few in low concentration.

We wish to thank Dr. R. M. Simington for suggestions and criticism and for supplying us with various dyes. We also wish to express our gratitude to the National Anilin and Chemical Company who furnished us liberal samples of many of their dyes.

CONCLUSIONS

1. Of 90 dyes which have been injected intravenously into animals with permanent pancreatic fistulae, 10 are eliminated in the pancreatic secretion. Sodium thiocyanate is eliminated, sodium iodide and pyridium are not.

2. The excreted dyes differ from each other markedly in chemical structure and physical properties. There appears to be no explanation on physical or chemical grounds for the elimination of these dyes and the retention of others.

3. Those dyes which are excreted appear in the pancreatic juice in concentrations very much lower than their probable concentration in the body fluids.

4. No dye is eliminated in sufficient quantity to be of practical value as a pancreatic function test.

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CARBOHYDRATE METABOLISM IN PARATHYROIDECTOMIZED DOGS[1]

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In a preliminary report (1) it was shown that complete parathyroidectomy does not affect fasting glucemia or dextrose tolerance except when animals are in tetany. It is the purpose of this paper to report the results of further experiments which suggest an involvement of carbohydrate metabolism in the production of tetany.

A review of the earlier literature on the relation of carbohydrate metabolism to parathyroid deficiency was made by Barker (2). Wishnofsky (3a), in a more recent review, has considered the rôle of calcium in carbohydrate metabolism. Martin (4) reviewed the literature on the nature of tetanic spasms and concluded that calcium deficiency facilitated hydrolysis of lactacidogen, thus producing an intrinsic or myogenic stimulus to contraction, basing his conclusion on the work of Lange (5) who found that both calcium and glycogen facilitated synthesis and inhibited decomposition of this body, confirming the results of Embden and his colleagues. Adam (6) also confirmed this point. Numerous investigators have found that ingestion of either sugar or phosphates increases the lactacidogen content of muscle.

Brougher (7), Dragstedt (8) and Salvesen (9) have each given reviews of literature bearing on the changes of calcium, phosphorus and sugar, much of which indicates an intimacy of relationship.

Kauffman-Cosla (22) holds that calcium is an indispensable factor in oxidation of sugar. Abelin (10) and Löb (11) believe phosphorus ions favorable and necessary in carbohydrate metabolism. Wishnofsky (3b) concluded that the parathyroids are not involved in carbohydrate metabolism. Decreased tolerance to ingested dextrose in parathyroid deficiency has been reported by Eppinger, Falta and Rudinger (2), Marine (2), Salvesen (9), Takahasi (12), Parhon and Décévisi (13) failed to confirm this. Calcium administration restores sugar mobilization (14). No one has reported hypoglucemia in human tetany. Hyperglucemia during tetany was reported by Magenta (15). Dextrose ingestion has been found

[1] This investigation was begun at Baylor Medical School, contined at the University of Chicago during the Summer Quarter of 1928 and completed in this Laboratory.

to produce hypophosphemia by Chang (15), Jost (17), Wigglesworth, Woodrow, Winter and Smith (18), Harrop and Benedict (19), Lambie and Redhead (20) (ingestion of dihydroxyacetone). Barrenscheen, Eisler and Popper (21) found first an increase followed by a decrease of both total and inorganic fractions. This is not due to increased renal elimination (16), (20), (21).

Adam (6) found that tetany could be relieved by sugar ingestion. He also believed that disturbances in carbohydrate metabolism in both rickets and tetany were indicated by increased diastase elimination. The hypophosphemia induced by sugar ingestion is less marked in diabetic patients (16). Behrendt (23) claimed that there is no decrease in lactacidogen content of the muscle of dogs in tetany, nor any change in calcium content. Shirley (24) has confirmed the often reported stimulant action of ingested phosphates. Greisheimer (25) has demonstrated that the withdrawal of sugar is of itself an augmentor of reflex irritability, and vice versa.

In addition to the citations given, there is a large amount of literature less directly related to the immediate problems, showing numerous relations of calcium, phosphorus and sugar in varying combinations, from which it is more and more apparent that there is an important interrelation which, perhaps, has not been fully appreciated heretofore.

Interest in this problem was first aroused by the work which Sweeney and Lackey (26) were carrying on in the same laboratory, in which it was shown that toxemias produce a characteristic curve of sugar tolerance. Owing to the apparent toxic condition in certain parathyroidectomized dogs, it was determined to investigate the curve of tolerance in these animals. The experiments originally reported have now been extended to include 15 operated dogs. Thirty-two observations were performed on 18 normal dogs, 17 of which were subsequently operated. Two of these died before any post-operative observations were made. Forty observations have been made on operated animals not in tetany, and 33 while in tetany. Two grams of dextrose per kilo of body weight were given orally after the initial blood samples were drawn. Observations extended over two hours in all cases, often three or four hours, though in all dogs not in tetany, effects were completed in from two to three hours. When in tetany the action was usually prolonged considerably, hyperglucemia sometimes persisting until the following day, if the animals were not treated in any other way, and were not relieved rather promptly by sugar. Hypoglucemia was not found consistently in any operated animal. When tetany occurred there was found a very characteristic curve of decreased tolerance, as shown in figure 1. In only four instances did a dog in tetany give a curve like that in normal animals. At present no explanation of this variation is possible. The absolute level of glucemia at the highest point varied greatly among normal, non-tetanic and tetanic dogs and in

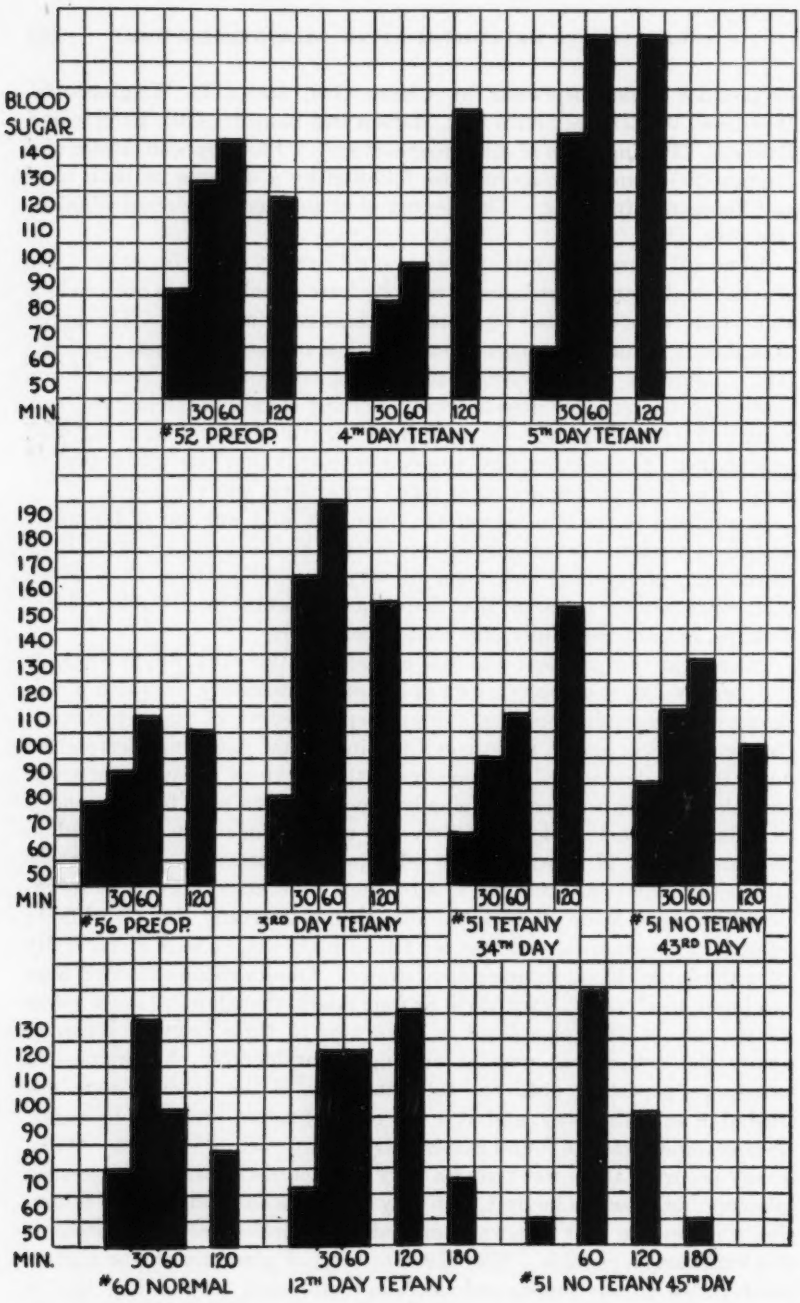

Fig. 1. Ordinate, milligrams dextrose per 100 cc. blood. Abscissa, time in minutes after oral administration of 2 grams dextrose per kilo of body weight.

TABLE 1

ANIMAL	TIME AFTER INGESTION	BLOOD SUGAR	PLASMA CALCIUM	INORGANIC PLASMA PHOSPHORUS	Ca:P	REMARKS
	minutes					
No. 56		92	11.58	5.40	2.14	Normal animal
	30	95	11.52	4.85	2.37	
	60	114	11.55	4.42	2.62	
	120	110	11.52	4.42	2.60	P—18 per cent
3rd day		84	6.42	7.40	0.86	Severe tetany
post	30	170	6.07	7.01	0.85	
opera-	60	198	6.66	7.14	0.93	
tive	120	160	5.96	6.25	0.95	Not entirely abated
			7%	−15.5%		
53rd day		93	5.08	5.65	0.89	Mild tetany
	30	129	5.08	5.23	0.97	
	60	132	6.10	4.30	1.42	
	120	123	5.33	4.90	1.08	Completely abated
			+4%	−13.5%		
No. 57		95	12.10	7.40	1.60	Normal dog
	30	137	12.17	6.40	1.76	
	60	154	12.22	6.06	2.01	
	120	127	11.64	5.54	2.10	
			3.8%	7.48%		Died in tetany 3rd day
No. 60		80	11.82	5.30	2.20	Normal
	30	138	11.26	5.03	2.20	
	60	103	11.22	5.42	2.07	
	120	87	11.10	5.40	2.05	
12th day		72	7.98	9.90	0.80	Tetany
	30	125	7.98	9.01	0.88	
	60	125	7.41	7.85	0.94	
	120	142	7.02	5.47	1.27	Completely relieved
	180	74	7.63	6.00	1.27	
24th day		93	7.48	4.08	1.83	Good condition
	30	113	7.48	3.65	2.05	
	60	111	7.41	3.26	2.27	
	120	100	7.02	3.22	2.14	
No. 51		96	6.33	7.69	0.8	Tetany
22nd day	30	116	6.29	5.00	1.06	
	60	137	6.20	4.73	1.30	
	120	170	6.10	4.28	1.40	Abated
33rd day		67	6.54	8.05	0.80	Tetany
	30	109				
	60	125				
	120	131	6.13	6.30	1.02	Relieved
34th day		71	7.30	7.30	1.00	Tense, fine tremor
	30	101				
	60	125				
	120	159				

TABLE 1—*Concluded*

ANIMAL	TIME AFTER INGESTION	BLOOD SUGAR	PLASMA CALCIUM	INORGANIC PLASMA PHOSPHORUS	Ca:P	REMARKS
	minutes					
43rd day		91	8.98	7.59	1.17	No tetany
	30	119				
	60	138				
	120	104				
45th day		60	9.78	6.21		No tetany
	60	149				
	120	103	12.25	5.65		
	180	60	12.81	7.01		
No. 57		81	5.70	6.72	0.84	Tetany
18th day	30	109	6.00	6.72	0.89	
	60	126	5.80			
	120	160	5.60	5.33	1.05	Improved

the same animal at different times. The only characteristic feature was the rate of reduction of hyperglucemia.

Since tabulation of total results would require too much space, a few typical figures are given in table 1, together with certain of the least typical in order to show the extent and type of variation.

It was usually unnecessary to draw more than 15 cc. of blood. Simultaneous determinations of calcium, phosphorus and sugar in heparinized plasma were made as in an earlier paper (27). However, it was possible to confirm the contention of Schimmelpfeng (28) that calcium precipitation should be prolonged 24 hours instead of 30 minutes to one hour as is usually done.

Systemic effects of tolerance tests. Animals in any stage of tetany were always relieved to some extent, usually completely. The degree of relief was in no way correlated with any observed factor. The same dog was completely relieved from a severe attack and subsequently, in a mild attack, experienced a much smaller degree of relief. All secondary symptoms were relieved parallel to the accompanying tetany. The well-known beneficial effects of a carbohydrate diet for parathyroidectomized animals have been ascribed to alterations in the intestinal flora (Dragstedt), or to improvement of absorption of calcium (29).

In view of the results of our experiments it has seemed probable that the beneficial effects may as well be ascribed, in part, to some intrinsic influence, since the tests were made on fasting dogs. As shown in the illustrations, there was definitely decreased tolerance only during an attack of tetany, or in a condition of generalized spasticity. The form of the graphs of hyperglucemia at once suggests that the condition of tetany

delayed absorption of ingested sugar. In order to test this possibility, intravenous injections of dextrose were made in ten experiments on dogs in tetany. In form, the curves obtained could not be distinguished from those following oral administration. Therefore, the effect is due to some intrinsic factor.

Dextrose treatment of parathyroid tetany. The above results suggested an effort to protect animals completely by intravenous injections of dextrose. Adams' (6) results were not known at that time. Sixteen dogs were operated in groups of four. Two in each group were not treated and all died in 31 to 77 hours. Beginning 20 hours after operation, the others were injected with 50 per cent dextrose solution, sometimes amounting to 40 grams, in the course of a day, in divided doses, the frequency and the total amount depending on the condition of the animal. Attacks of tetany were frequent and were not always abated by the treatment, but no other was employed. In this connection it should be mentioned that all dogs used in tolerance tests were treated with parathormone at various times.

At the end of fourteen days dextrose injections were discontinued on four dogs, all of which died of tetany within 50 hours. At the end of twenty-five days treatment was discontinued on the other four, in three of which no attacks of tetany were noted after the 20th day. No further attacks occurred but all died in depression on the 31st, 31st, 34th and 37th days respectively. After the fifteenth day all of these dogs showed symptoms of spasticity which became progressively worse until they could walk only with difficulty, particularly after resting in the cages for some time. There were frequently noticed orthotonic contractures, at other times asymmetric contractures resulting in lateral curvature so that progression was circular or spiral. However, none of these were in any apparent distress, taking food and water freely. No blood or urine analyses were made, the purpose being to determine if these measures would protect during the acute stage. The early death after the discontinuance of treatment to the third group and the prolongation of life in the fourth group indicate positive protection. The procedure is not important as a routine protective measure but does constitute further indication of relationship between the tetanic condition and carbohydrate metabolism.

Calcium and phosphate. Simultaneous determinations of dextrose, calcium and inorganic and total phosphorus of the plasma, and in a few instances, of total and inorganic blood phosphorus, have indicated important relationships among these constituents. While there was never a very close parallel in the changes noted among these elements, nevertheless certain general tendencies may be noted. In normal and non-tetanic dogs calcium was usually diminished slightly, though in two instances there was a slight increase and a subsequent fall so that the ultimate result

was a slight decrease at the end of two hours. In one case there was practically no change. At three hours there was always restoration to practically the initial value. When in tetany the calcium response was quite irregular but there was always a pronounced fall somewhere in the course of the experiment, except in two instances, one of which is shown in the table. In the other, there was a rise from 9.78 mgm. to 12.25 mgm. at the end of two hours and to 12.81 mgm. in three hours. In all other cases the final result was a decrease at the end of two hours.

Total and inorganic blood phosphorus and total plasma phosphorus were decreased in all cases but the number of determinations is too small to permit any further conclusions. Inorganic plasma phosphorus was decreased progressively in all normal animals except in two experiments, in both of which there was a slight fall followed by a slight increase. In operated animals not in tetany there were no exceptions. In tetany there was always a decrease slightly greater in absolute amount than in the other two groups.

The importance of the Ca:P ratio has been emphasized in another paper (27). In this series, the ratio was always increased progressively in operated animals, regardless of the fluctuations of the concentrations of the two elements. In all cases without exception, where the symptoms were ultimately abated, the ratio was increased above one. The relation of this factor to the presence of tetanic symptoms is therefore of considerable importance.

In general, there was a greater irregularity of response in all factors to the ingestion of sugar after three to four weeks, than was apparent in the early post-operative period. Possibly this fact is related in some way to latent tetany.

The importance of the interrelationships of calcium and phosphorus has been shown in many ways. While there is no demonstrable direct relation between calcium and dextrose, that between dextrose and phosphorus has been clearly indicated in many ways, particularly in the many investigations on muscle metabolism. The importance of carbohydrate metabolism in parathyroid deficiency has been shown by numerous dietary experiments. This paper offers further indication of the importance of this relationship. A survey of all available evidence, then, prompts the suggestion that the phenomena of parathyroid deficiency involve a much more complicated mechanism than is ordinarily indicated and that the solution of the problem will not be arrived at by the investigation of any one factor alone but by consideration of the whole complex scheme of interrelationships among constituents of blood and muscle. The connecting links are by no means clear as yet. But the evidence is strongly indicative of the direction in which further investigation of this problem must proceed. Morris (30) and Campbell (31) have shown that the one

condition common to all types of tetany is anoxemia and that tetany relieves this condition, also that anoxemia is the fundamental cause of neuromuscular excitability. Should this prove correct, the necessity of carbohydrate involvement in tetany is still more evident.

It is acknowledged that this investigation brings out no very pronounced relationship but it must be remembered that the blood changes are not always absolute indices of conditions in the tissues. It seems that parathyroid tetany is of myogenic origin. In that case the ultimate solution of the problem will be found in investigations of muscle metabolism.

SUMMARY

The response of normal and parathyroidectomized dogs to the ingestion of 2 grams of dextrose per kilo of body weight has been investigated.

1. Dextrose tolerance curves show a characteristic response during attacks of tetany, indicating decreased tolerance.

2. Symptoms are abated after dextrose ingestion or injection.

3. Tolerance tests by intravenous injection give identical results, therefore, an intrinsic mechanism is involved.

4. Intravenous injections of concentrated dextrose solutions will protect dogs during the acute stage and prolong life but will not prevent the appearance of myotonic symptoms.

5. During tolerance tests the following changes in blood constituents occurred:

a. Calcemia was usually slightly decreased in normal, and non-tetanic animals. In tetany the decrease was slightly greater.

b. Phosphemia was generally decreased in normal and non-tetanic animals. In tetany there was always a more pronounced decrease.

c. The Ca:P ratio was always increased. When symptoms were completely abated the final figure was above one.

6. These results are believed to indicate an involvement, possibly indirectly, of carbohydrate metabolism in tetany.

7. The fasting level of glucemia was not affected by the operation or the occurrence of tetany.

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THE EFFECTS OF INSULIN ON PARATHYROIDECTOMIZED DOGS

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In the accompanying paper the relation of carbohydrate metabolism to tetany was discussed. It is the purpose of this paper to report the results of the use of insulin in normal and parathyroidectomized dogs. Brougher (1) has reported that in both normal and parathyroidectomized dogs insulin produced hypercalcemia, a result which Culhane (2) failed to confirm for normal rabbits. Magenta (3) found that dogs in tetany were more sensitive to insulin, a result which he concluded to be due to the state of tetany rather than to parathyroid deficiency per se. Either hypophosphemia or hypoglucemia, or both, as a result of insulin injection, have been found by Chaikoff, Macleod, Markowitz and Simpson (4), Florence and Zola (5), and Kerr (6). The latter found this action confined to the serum content. Barrenscheen, Eisler and Popper (7) claim that insulin does *not* influence the phosphorus curve but does inhibit phosphate elimination. Simultaneous determinations of blood sugar, calcium and inorganic phosphorus were made at intervals of one hour after subcutaneous injection of insulin in varying doses. Observations extended over periods of two hours in all cases, often over three hours.

The results are shown in table 1. Six out of sixteen experiments on normal dogs were selected at random for tabulation; fourteen observations were made on operated dogs not in tetany, of which four are tabulated; five out of twelve observations on dogs in tetany are included in the tables.

In normal animals, typical blood sugar curves were obtained. Dog 69, in the second experiment, showed typical insulin convulsions just before the third observation was made. In all cases except one (dog 68, second experiment) calcemia was increased progressively though the curves were by no means uniform. The increase was between 4 per cent and 15 per cent, averaging approximately 10 per cent. All factors showed a tendency to recovery after two hours, confirming the report of Brougher (1).

Inorganic phosphorus was decreased in all cases, the greatest decrease occurring in the first hour except in one case, the same one which showed the atypical response for calcium. The range of decrease was 23 per cent to 58 per cent, average 30 per cent.

TABLE 1

NUMBER	HOURS	BLOOD SUGAR	CALCIUM	INORGANIC P	Ca:P	REMARKS
308		95	10.53	6.55		6 units insulin subcutaneously
	1	63	12	5.88		
	2	52		4.10		
68		87	10.71	8.60		10 units insulin subcutaneously
	1	50	10.94	5.80		
	2	41	11.18	5.40		
	3	57	11.94	6.45		
		79	11.99	8.16		30 units insulin subcutaneously
	1	48	11.58	7.82		
	2	43	13.40	3.48		
		96	10.62	5.80		25 units
		60	11.66	4.68		
		66		4.20		
69		80	10.56	4.44		50 units
	1	43	10.62	4.00		
	2	35	12.03			
		79	10.43	7.40		20 units
	1	35	11.37	5.35		
	2	25		4.27		
Operated dogs						
60		88	11.80	4.00	2.90	10 units. No tetany
51st day	1	76	10.60	3.65	2.90	
	2	40	11.60	2.85	4.00	
91st day		74	9.78	4.62	2.10	35 units. No tetany
	1	40	9.40	2.85	3.30	
	2	33	10.43	1.70	6.10	
64		83	10.81	5.83	1.85	15 units. No tetany
2nd day	1	66	11.07	4.62	2.39	
	2	52	11.58	3.91	2.96	
66		82	12.10	3.77	3.20	8 units. No tetany
5th day	1	55	12.90	3.24	4.00	
	2	44	13.30	2.66	5.00	
8th day		80	6.80	6.95	9.97	10 units. No tetany
	1	53	7.14	5.40	1.32	Symptoms abated
	2	49	6.30	5.53	1.12	
63		79	6.31	7.47	0.84	10 units. Tetany
32nd day	1		6.77			Symptoms abated
	2	52	6.23	4.96	1.25	
	3	51	6.00	5.12	1.17	

TABLE 1—*Concluded*

NUMBER	HOURS	BLOOD SUGAR	CALCIUM	INORGANIC P	Ca:P	REMARKS
41st day		96	4.89	7.55	0.64	Tetany. 15 units
	1	72	4.17	7.40	0.56	Not relieved
	2	48	4.83	5.36	0.86	
45th day		84	6.04	8.16	0.74	15 units. Tetany
	1	51	11.36	7.01	1.61	Symptoms abated
	2	87	7.20	5.16	1.39	
64		80	7.62	7.81	0.97	15 units. Tetany
7th day	1	57	6.41	6.63	0.86	
	2	47	7.90	4.13	1.90	Symptoms abated

The extent of change in both calcium and phosphorus was not closely correlated with the size of the dose of insulin nor with the absolute initial concentration. The Ca:P ratio was always increased.

In operated animals not in tetany, the responses of all these constituents did not vary particularly from those found in normal animals.

In tetany the fasting level of glucemia was not reduced nor could any peculiarity of response to insulin be found. Calcemia responded much less regularly than in the other groups. There was usually an increase at some point followed by a decrease. In two cases there was a decrease followed by a rise, in one, above the initial level.

Phosphemia was decreased in much the same manner as in the other groups except perhaps somewhat less regularly. The Ca:P ratio was always increased. In all cases when the tetanic spasm was abated the ratio was greater than one. In two instances relief was not obtained and it was necessary to give parathormone. In one of these, dog 63 on the 41st day after operation, 15 units did not give relief, while 10 units on the 32nd day relieved completely an attack of approximately the same degree of severity, though hypoglucemia was of a greater degree on the later date. Phosphemia was approximately the same which, of course, reduced the ratio in the later attack.

After an attack of tetany that was abated by insulin, animals were free from attacks somewhat longer than when, by parathormone, an equivalent degree of relief was obtained as indicated by approximately the same level of calcemia and phosphemia. This fact suggests that the additional protection was afforded by the effect of insulin on carbohydrate metabolism. Whether animals could be protected as long by repeated insulin injections was not determined. These facts indicate that the results reported in the accompanying paper are not due primarily to increased insulin secretion stimulated by hyperglucemia because the

general response of calcium to hyperglucemia was a decrease, and to injected insulin, an increase.

SUMMARY

Insulin administration to normal and parathyroidectomized dogs not in tetany will increase calcemia and decrease phosphemia. When in tetany, insulin will relieve symptoms, usually completely, and will produce hypophosphemia. Calcemia responds much less regularly than in normal animals, any increase being followed by a decrease. Tetany alters the effect of insulin on calcemia and to a less extent on phosphemia, but does not affect the post-insulin hypoglucemia curve.

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THE REGULATION OF RESPIRATION

XXXI. The Effect of Hemorrhage, Reinjection, and Low Alveolar Oxygen on the Reflex Activity of the Cord

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The experiments described in this paper were undertaken in order to compare the effects of hemorrhage, reinjection, and low alveolar oxygen on the activity of the respiratory center and of the lower spinal reflex centers. It was hoped that such a study would help to elucidate the mechanism of the control of respiration.

Method. The reflex chosen for study was the contraction of the tibialis anticus muscle of the dog. This has a simple reflex arc involving one sensory and one motor neurone (1). The procedure followed is briefly described. After the dog was anesthetized with morphine and urethane, the tibialis anticus tendon was isolated, severed at its insertion, and attached to a muscle lever to record its contractions, the leg itself being firmly attached to fixed horizontal bars. Usually one muscle was prepared in this way, but in some experiments the tibialis anticus of the other side was isolated and attached in the same manner. The purpose of this will be described below. The reflex was usually elicited by unipolar stimulation, a large indifferent electrode being attached to the neck and a small stimulating electrode inserted in the skin of the foot. In some experiments the bipolar method was used, the sciatic nerve being isolated on both sides, and a pair of electrodes placed on the motor branch on one side and on the sensory branch on the other. The stimuli used were shocks from an induction coil whose primary circuit contained a device for making and breaking the circuit at regular intervals. Break shocks only were used, the make shocks being eliminated by the use of a power tube as rectifier.

The respiration was recorded by connecting the trachea with a closed circuit rebreathing tank containing a soda lime cartridge to absorb carbon dioxide. Two such tanks were used, the one containing room air and the other a mixture of oxygen in nitrogen (usually about 8 per cent oxygen). The apparatus was so arranged that it was possible to shift from one to the other at will. Blood pressure was recorded from the carotid artery. Blood was drawn either from the femoral artery or from the other carotid

[1] Assisted in the experimental part of the work by Mr. C. W. Walton.

and injections were made either into the femoral or into the external jugular vein.

This method is substantially that of Glazer (1929). The amplitude of the response of the tibialis anticus muscle remains approximately constant for a considerable length of time when the muscle is stimulated reflexly by submaximal induction shocks of constant strength, at regular intervals of several seconds under constant conditions. Accordingly it was assumed that any marked changes observed in the height of the contractions, following hemorrhages and other experimental procedures, were due to significant changes either in the central synapse or in the peripheral nerve

TABLE 1

Hemorrhages

NUMBER OF DOGS ON WHICH DATA ARE BASED	SIZE OF HEMORRHAGE	TOTAL NUMBER	REFLEX			RESPIRATION		
			Decrease	Unchanged	Increase	Decrease	Unchanged	Increase
	per cent							
8	1.00	16	1	9	6	1	8	7
20	1.00, 2.00	48	4	15	29	5	11	32
5	2.00	10	1	2	7	1	0	9
Total 29		74	6	26	42	7	17	48

Injections

NUMBER OF DOGS ON WHICH DATA ARE BASED	SIZE OF INJECTION	TOTAL NUMBER	REFLEX			RESPIRATION		
			Decrease	Unchanged	Increase	Decrease	Unchanged	Increase
	per cent							
6	1.00	8	5	3	0	2	5	1
21	1.00, 2.00	49	23	16	10	19	23	7
4	2.00	5	3	1	1	3	2	0
Total 29		62	31	20	11	24	30	8

and muscle. Attempting to differentiate central and peripheral effects a special series of experiments using the bipolar method of stimulation, as mentioned above, was carried out. In this experiment contractions of the tibialis muscle of one leg, stimulated reflexly, and of the other leg, stimulated by its motor nerve, were recorded simultaneously and the results compared.

RESULTS. The effects of hemorrhage and of the reinjection of defibrinated blood or glucose or gum saline solutions were studied in twenty-nine experiments. In the course of these experiments 74 hemorrhages and 62 reinjections were performed. The results are summarized in table 1.

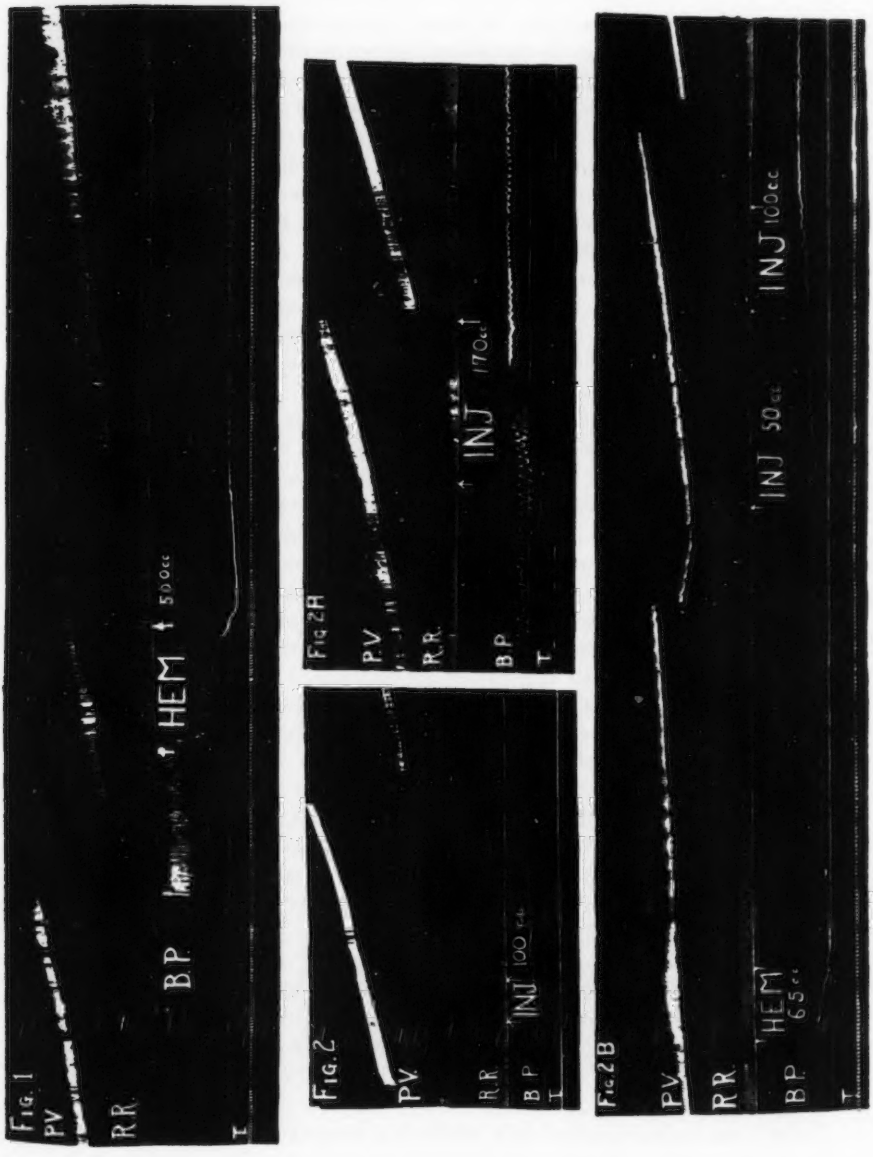
FIG. 1
PV
RR
BP
HEM 50cc
T
FIG. 2
PV
RR
BP
INJ 100 cc
T
FIG. 2A
PV
RR
BP
INJ 170cc
T
FIG. 2B
PV
RR
BP
HEM 65cc
INJ 50cc
INJ 100cc
T

Figs. 1-2B

It will be seen that the results varied wth the animal and with the size of the hemorrhage and injection. In some cases no change at all took place, as for example following the small hemorrhage shown at the beginning of figure 2B, or the larger hemorrhage shown in figure 9; but in general hemorrhage produced an increase in the amplitude of the reflex contraction (fig. 1, 3, 4, and 5), while reinjection, whether of glucose saline, gum saline, or defibrinated blood, produced a decrease in amplitude (fig. 2, 2B, 4). Occasionally, however, a divergence from these generally observed results was noted. At times hemorrhage was followed by a decrease, while not infrequently reinjection produced an increase in amplitude (fig. 2A). The table will serve to show the relative frequency with which these effects were obtained. If in this table we group together all the hemorrhages and similarly all the injections producing some definite change, we find that 87.5 per cent of the former caused an increase in reflex response, while 73.8 per cent of the latter produced a decrease. We may also note in passing that the table shows that the larger the hemorrhage or injection the more often was a positive result obtained.

An examination of the individual records reveals the fact that there were certain differences in the manner—abrupt or progressive, permanent or transient—in which the increase or decrease took place. Thus in figure 1 the reflex response began to increase during the hemorrhage, continued to grow progressively for a short interval thereafter, and then, as the blood pressure spontaneously rose, the reflex fell off till it reached a level below that preceding the hemorrhage. At the very end of figure 1 we see the blood pressure beginning to fall again after the temporary rally, and coincident with this final fall the reflex response is beginning to rise a little. Figure 3, on the other hand, instead of such progressive changes, shows an abrupt rise during the hemorrhage, followed by a more immediate return to normal. Figure 4 shows an experiment in which the increase following the hemorrhage was not so transient as in figures 1 and 3, but continued higher than normal, until a reinjection brought about a decrease. Similarly, in the case of injections, we find in figure 4 an abrupt and permanent fall, in figure 2 a progressive and permanent fall, and in figure 2B a progressive but transient drop during and just after the injection, followed by a partial return to normal (at the very end of the record). Figure 2 also shows a case in which a definite latent period intervened before the effect of the injection became apparent. Moreover the magnitude of the change in the blood pressure was found to vary considerably; for example, in figure 4 the change in blood pressure is very large, in figure 2 comparatively insignificant.

Since the main object of this study is a comparison of the effects of hemorrhage and reinjection on respiration and on reflex activity, it is

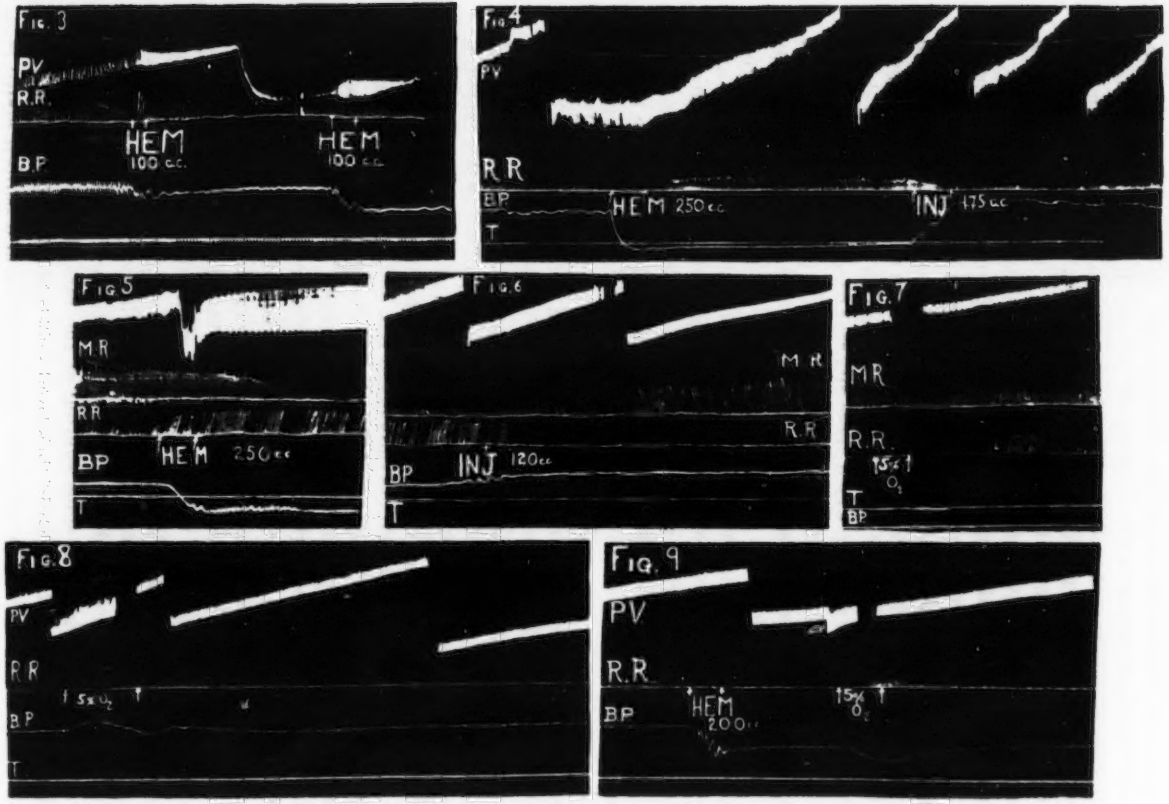
Fig. 3
PV
R.R.
B.P.
HEM 100 c.c.
HEM 100 c.c.
Fig. 4
PV
R R
BP
HEM 250 c.c.
INJ 175 c.c.
T
Fig. 5
M R
R R
BP
HEM 250 c.c.
T
Fig. 6
M R
R R
BP
INJ 120 c.c.
T
Fig. 7
M R
R.R.
15% O2
T
BP
Fig. 8
PV
R R
15% O2
B.P.
T
Fig. 9
PV
R.R.
BP
HEM 200 c.c.
15% O2

Figs. 3-9

important to examine the records with reference to a possible correlation. The records show that hemorrhage usually produced, for a time at least, an increased pulmonary ventilation (figs. 1, 3, 5), often followed by a depression (fig. 2B), while reinjection usually produced a decreased pulmonary ventilation (fig. 2), when it had any effect at all. There was, however, a certain amount of divergence from these usual results from time to time, as may be seen from table 1 given above, as well as from the records. Figure 6, in which the injection caused a slight increase in the respiration, at the same time that it depressed the reflex response, is one of these abnormal cases. The somewhat variable effects on respiration confirm the findings of Bald (1927) and of McGinty and Gesell (1928) on this point. Table 1 shows the relative frequency with which these different effects on respiration were obtained. Grouping together all cases in which a definite change in the pulmonary ventilation was observed, we find that 87.3 per cent of the hemorrhages produced an increase while 75 per cent of the injections produced a decrease. The corresponding figures for the reflex response are, as given above, 87.5 per cent for the hemorrhages followed by an increase, and 73.8 per cent for the injections followed by a decrease. It is accordingly evident that from the statistical point of view the correlation is very close. A study of the individual records is, however, necessary to establish the existence of a true parallelism; and turning to the records we find that in point of fact they do show a close correlation between changes in the respiration and those in the reflex response. Figures 1, 2, 2A, 3, and 5 are cases to the point. Thus in figure 1 the pulmonary ventilation is increased during hemorrhage, first in rate and then in depth; following this there is a period of depression, and finally an increase again at the end of the record. These changes correspond rather closely to the increase of the reflex response during hemorrhage, its subsequent fall, and its final rise. Figure 2 shows a gradual and permanent decrease in the pulmonary ventilation, accompanying the depression in the reflex response. Figure 2A, the record of the exceptional rise in the reflex response following an injection, nevertheless shows a very clear simultaneous and parallel increase in the pulmonary ventilation, and accordingly is an excellent instance of the correlation between the two. Figure 3 shows an increase in the pulmonary ventilation with each of the hemorrhages returning rapidly to normal afterwards. These changes correspond closely to the simultaneous transitory increases in the reflex response. Figure 5 shows an example of a hemorrhage which was followed by a great increase in the depth of the respiration, together wth some slowing of the rate; but the total pulmonary ventilation is evidently increased, and this increase runs parallel with the increase in reflex activity. This correlation described above is not quite universal in every experiment, for at times the reflex and the

respiration varied independently or even in divergent directions. But such simultaneous augmentation following hemorrhage and simultaneous depression following injection were seen most frequently. One point worthy of notice is the fact that the respiratory center appeared to be more sensitive to small changes in the blood flow than was the reflex arc. Results similar to those recorded in figure 2B, in which a small hemorrhage left the reflex response practically unchanged but greatly increased the respiration, were not uncommon.

The administration of low percentages of oxygen in nitrogen was found to give a marked rise in the amplitude of the reflex contraction (figs. 7, 8, 9). The same animals that were used for the hemorrhage experiments were also used for these; low oxygen was administered at various times during the experiments, and its stimulating effect was usually as readily obtainable after the animal had been somewhat weakened by hemorrhage as before (fig. 9). Indeed the effect was normally quite marked and was obtained with extraordinary constancy; mixtures containing less than about 10 per cent oxygen produced the increase sooner or later. The few times in which increase was not apparent occurred only when the time of administration was very short, or when toward the end of the experiment the animal had lapsed into bad condition. The pulmonary ventilation almost always increased during administration, normally parallel with the increase in the reflex (figs. 8 and 9). Occasionally it became periodic. No constant correlation between the periodicity of the respiration and that sometimes observed in the reflex could, however, be made clear. The administration of gaseous mixtures lower in oxygen was usually accompanied by a change in blood pressure (figs. 8 and 9) showing no correspondence with the change in reflex.

It is of interest to know to what extent the changes in amplitude of the reflex response are peripheral or central in character. With that in mind a few experiments were performed including simultaneous study of the response to direct stimulation of the motor nerve fibers and to indirect stimulation through the sensory nerve fibers. In these experiments small hemorrhages and reinjection usually had no effect on the direct motor response, large hemorrhages usually produced a decrease (fig. 5) and large injection sometimes produced an increase (fig. 6). In the limited number of experiments performed the reverse effects were never observed. These changes in direct motor response were in the opposite direction to those observed in the reflex response recorded in the same animal during the same period. The significance of these results will be discussed later.

In a similar way simultaneous records were made of the direct and indirect response during the administration of low oxygen mixtures. Only a few such experiments were done. In none of these was the direct response

effected though the reflex response showed the usual augmentation (fig. 7). That this absence of change in the direct motor response, however, is not a constant one has been shown by the more recent experiments of Gay (unpublished). He finds a frequent increased response to stimulation of the motor nerve on the administration of low oxygen mixtures.

Discussion. From the work of Gesell and others the effects of hemorrhage and reinjection on the volume flow of blood are well known. Hemorrhage not only decreases the flow of blood through individual tissues but apparently through the body as a whole. It is accordingly assumed that hemorrhage reduced the flow of blood through spinal cord and brain. It is thus safe to assume an acid base disturbance in the tissues as a result of hemorrhage as well as a curtailment of oxidations. Reinjection of an inert solution would, by the same line of reasoning, tend to increase the oxygen supply and ultimately if not immediately diminish the hydrogen ion concentration of the tissues. The reflex response to hemorrhage might, therefore, be a resultant of two factors—a diminished supply of oxygen and an accumulation of acid metabolites. Glazer (1929) found that breathing of gas rich in carbon dioxide, which would favor the accumulation of acid metabolites in the tissues depressed the reflex response, and that sodium cyanide injections, which are known to paralyze oxidations, increased the reflex response; and we have found, as described above, that the anoxemia produced by low alveolar oxygen augmented the reflex. Accordingly, if we conclude from these observations that lack of oxygen tends to stimulate the reflex and excess of carbon dioxide and metabolites tends to depress it, then it must follow that the effects of hemorrhage or reinjection should be the resultant of these two forces acting in opposite directions. As a matter of fact the results obtained support this line of contention that there is a possible dual effect, as evidenced by the somewhat variable character of the results and by the large number of cases in which no change occurred even after large hemorrhages and reinjections. The variability of the effects of hemorrhage thus agrees with the variability of the effect of mechanical asphyxia described by Glazer (1929).

It is obvious that the source of changes in the reflex response during hemorrhage or during injection may be in any part of the reflex arc from the afferent fibers to the end organ. In his studies on mechanical asphyxia and administration of sodium cyanide Mr. Glazer made no effort to distinguish between central and peripheral effects. Our limited study of the response of the tibialis anticus to direct stimulation of the motor nerve fibers and to reflex stimulation is a beginning of such an attempt. As stated before, hemorrhage, if it affected the direct motor response at all, tended to decrease it, while reinjection tended to increase it. These results are in agreement with those of Gesell (1922) for tetanic stimulation. Accordingly, since these effects may be just the reverse of those simul-

taneously observed in the reflex response, they would tend to oppose and so mask the changes produced in the reflex, rather than be in any way responsible for them.

With respect to the explanation of the augmentation of the reflex response by low alveolar oxygen our experiments indicate that they may be independent of any change in the motor nerve, motor end plate and muscle. Mr. Gay's experiments (unpublisned), however, indicate that the peripheral motor mechanism may frequently be a source of alteration of reflex response. For the present it would seem advisable not to attempt definite conclusions in the localization of effect of hemorrhage of low alveolar oxygen on the reflex response.

With regard to a comparison of the effects of hemorrhage, reinjection, and low oxygen on the reflex studied and on the respiratory movements, we have seen the evidence of parallelism in all three cases. Whether we judge from the aggregate of the results collected in table 1, or from the individual records analyzed above, we must conclude that the activities of the respiratory muscles and of the lower spinal reflex arcs studied, may and in the majority of cases do vary in the same direction following hemorrhage, reinjection, and low alveolar oxygen followed by readministration of room air. Here too it is probably better not to enter into a discussion of the possible general significance of this correlation until further work has given us more insight into this problem. It will, however, be recalled that the administration of carbon dioxide may produce diametrically opposite effects on respiratory movements and spinal reflex response (Glazer, 1929).

SUMMARY

A series of experiments undertaken to investigate the effects of hemorrhage, reinjection, and low alveolar oxygen on the amplitude of the response of the tibialis anticus muscle of the dog to reflex stimulation is summarized. The method used for the study of variations in the reflex activity of the cord is described.

The effects of hemorrhage and reinjection varied with the animal and with the magnitude of the change in blood volume. The common result of hemorrhage was an increased reflex response, and that of reinjection a decreased response. Exceptions to these results and absence of effects are noted and discussed.

When the muscle was stimulated by its motor nerve, the reverse effects if any were observed. Hemorrhage was followed by a decrease and reinjection by an increase in amplitude of contraction.

Low alveolar oxygen produced an increase in the amplitude of the reflex response.

In the great majority of cases a definite parallelism was observed between the changes in the reflex response and the simultaneous changes in the pulmonary ventilation.

These results are discussed, and an attempt made to analyze the factors involved.

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